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Nucleic acid sequences encoding proteins associated with abiotic stress response

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## Nucleic acid sequences encoding proteins associated with abiotic stress response

5 This invention relates generally to nucleic acid sequences encoding proteins that are associated with abiotic stress responses and abiotic stress tolerance in plants. In particular, this invention relates to nucleic acid sequences encoding proteins that confer drought, heat, cold, and/or salt tolerance to plants.

10 Abiotic environmental stresses, such as drought stress, salinity stress, heat stress, and cold stress, are major limiting factors of plant growth and productivity (Boyer, 1982. *Science* 218, 443-448). Crop losses and crop yield losses of major crops such as rice, maize (corn) and wheat caused by these stresses represent a significant economic and political factor and contribute to food shortages in many underdeveloped countries.

15 Plants are typically exposed during their life cycle to conditions of reduced environmental water content. Most plants have evolved strategies to protect themselves against these conditions of low water or desiccation (drought). However, if the severity and duration of the drought conditions are too great, the effects on plant development, growth and yield of most crop plants are profound. Continuous exposure to drought causes major alterations in the plant metabolism. These great changes in metabolism ultimately lead to cell death and consequently  
20 yield losses.

Developing stress-tolerant plants is a strategy that has the potential to solve or mediate at least some of these problems (McKersie and Leshem, 1994. *Stress and Stress Coping in Cultivated Plants*, Kluwer Academic Publishers). However, traditional plant breeding strategies to develop new lines of plants that exhibit resistance (tolerance) to these types of stresses are  
25 relatively slow and require specific resistant lines for crossing with the desired line. Limited germplasm resources for stress tolerance and incompatibility in crosses between distantly related plant species represent significant problems encountered in conventional breeding. Additionally, the cellular processes leading to drought, cold and salt tolerance are complex in nature and involve multiple mechanisms of cellular adaptation and numerous metabolic pathways (McKersie  
30 and Leshem, 1994. *Stress and Stress Coping in Cultivated Plants*, Kluwer Academic Publishers). This multi-component nature of stress tolerance has not only made breeding for tolerance largely

unsuccessful, but has also limited the ability to genetically engineer stress tolerance plants using biotechnological methods.

Drought, heat, cold and salt stresses have a common theme important for plant growth and that is water availability. Plants are exposed during their entire life cycle to conditions of reduced environmental water content. Most plants have evolved strategies to protect themselves against these conditions. However, if the severity and duration of the drought conditions are too great, the effects on plant development, growth and yield of most crop plants are profound. Since high salt content in some soils result in less available water for cell intake, its effect is similar to those observed under drought conditions. Additionally, under freezing temperatures, plant cells loose water as a result of ice formation that starts in the apoplast and withdraws water from the symplast (McKersie and Leshem, 1994. *Stress and Stress Coping in Cultivated Plants*, Kluwer Academic Publishers). Commonly, a plant's molecular response mechanisms to each of these stress conditions are common.

The results of current research indicate that drought tolerance is a complex quantitative trait and that no real diagnostic marker is available yet. High salt concentrations or dehydration may cause damage at the cellular level during drought stress but the precise injury is not entirely clear (Bray, 1997. *Trends Plant Sci.* 2, 48-54). This lack of a mechanistic understanding makes it difficult to design a transgenic approach to improve drought tolerance. However, an important consequence of damage may be the production of reactive oxygen radicals that cause cellular injury, such as lipid peroxidation or protein and nucleic acid modification. Details of oxygen free radical chemistry and their reaction with cellular components such as cell membranes have been described (McKersie and Leshem, 1994. *Stress and Stress Coping in Cultivated Plants*, Kluwer Academic Publishers).

There are numerous sites of oxygen activation in the plant cell, which are highly controlled and tightly coupled to prevent release of intermediate products (McKersie and Leshem, 1994. *Stress and Stress Coping in Cultivated Plants*, Kluwer Academic Publishers). Under abiotic stress situations, it is likely that this control or coupling breaks down and the process "dysfunctions" leaking activated oxygen. These uncoupling events are not detrimental provided that they are short in duration and that the oxygen scavenging systems are able to detoxify the various forms of activated oxygen. If the production of activated oxygen exceeds the plant's capacity to detoxify it, deleterious degenerative reactions occur. At the subcellular level, disintegration of membranes and aggregation of proteins are typical symptoms. Therefore it is the balance between the production and the



scavenging of activated oxygen that is critical to the maintenance of active growth and metabolism of the plant and overall environmental (abiotic) stress tolerance.

Preventing or diminishing the accumulation of oxygen free radicals in response to drought is a potential way to engineer tolerance (Allen, 1995. *Plant Physiol.* 107, 1049–1054).  
5 Overexpression of antioxidant enzymes or ROS-scavenging enzymes is one possibility for the induction of functional detoxification systems. For example, transgenic alfalfa plants expressing Mn-superoxide dismutase tend to have reduced injury after water-deficit stress (McKersie et al., 1996. *Plant Physiol.* 111, 1177–1181). These same transgenic plants have increased biomass production in field trials (McKersie et al., 1999. *Plant Physiology*, 119: 839–847; McKersie et al.,  
10 1996. *Plant Physiol.* 111, 1177–1181). Transgenic plants that overproduce osmolytes such as mannitol, fructans, proline or glycine-betaine also show increased resistance to some forms of abiotic stress and it is proposed that the synthesized osmolytes act as ROS scavengers (Tarczynski. *et al.* 1993. *Science* 259, 508–510; Sheveleva, *et al.* 1997. *Plant Physiol.* 115, 1211–1219). Overexpression of glutathione reductase has increased antioxidant capacity and  
15 reduced photoinhibition in poplar trees (Foyer et al., 1995. *Plant Physiology* 109: 1047–57 ).

The glutaredoxin and thioredoxin proteins are small heat-stable oxidoreductases that have been conserved throughout evolution. They function in many cellular processes, including deoxyribonucleotide synthesis, protein folding, sulfur metabolism and most notably repair of  
20 oxidatively damaged proteins. They have also been implicated in the regulation of redox homeostasis in the cell and redox potential has been implicated in changes in gene expression.

Thioredoxins have a dithiol/disulfide (CGPC) at their active site and are the major cellular protein disulfide reductases. Cytosolic isoforms are present in most organisms. Mitochondria have a separate thioredoxin system and plants have chloroplast thioredoxins, which regulate photosynthetic enzymes by light via ferredoxin-thioredoxin reductase. Thioredoxins are  
25 critical for redox regulation of protein function and signaling via thiol redox control. Several transcription factors require thioredoxin reduction for DNA binding (Arner and Holmgren, 2000. *European Journal of Biochemistry* 267: 6102–6109; Spyrou et al., 2001. *Human Genetics* 109: 429–439).

Glutaredoxins are small heat-stable proteins that are active as glutathione-dependent  
30 oxidoreductases. They catalyze glutathione-disulfide oxidoreductions overlapping the functions of thioredoxins and using reducing equivalents from NADPH via glutathione reductase. In *Saccharomyces cerevisiae*, two genes, GRX1 and GRX2, whose expression is induced in response to various stress conditions including oxidative, osmotic, and heat stress, encode

glutaredoxins. Furthermore, both genes are activated by the high-osmolarity glycerol pathway and negatively regulated by the Ras-protein kinase (Grant CM. 2001. *Molecular Microbiology* 39: 533-541; Grant CM et al., 2001. *Biochimica et Biophysica Acta - Gene Structure & Expression* 1490: 33-42).

- 5 Another subfamily of yeast glutaredoxins (Grx3, Grx4, and Grx5) differs from the first in containing a single cysteine residue at the putative active site (Rodriguez-Manzanique et al., 1999. *Molecular & Cellular Biology* 19: 8180-8190). The role of these enzymes is not fully understood.

- 10 In addition to the two gene pairs encoding cytoplasmic glutaredoxins (GRX1, GRX2), *Saccharomyces cerevisiae* also contains two gene pairs for thioredoxins (TRX1, TRX2). Only a quadruple mutant is non-viable and either a single glutaredoxin or a single thioredoxin can sustain viability, indicating some cross function between the two systems (Draculic et al., 2000. *Molecular Microbiology* 36: 1167-1174).

- 15 Plants also contain glutaredoxins genes. A glutaredoxin (thioltransferase), which catalyzes thiol/disulfide exchange reaction, was isolated from rice (*Oryza saliva* L.) (Sha et al., 1997. *Journal of Biochemistry* 121: 842-848; Sha et al., 1997. *Gene* 188: 23-28; GenBank accession number D86744). Multiple forms of glutaredoxin have also been predicted in the *Arabidopsis* genome (GenBank).

- 20 Dehydroascorbate reductase (DHAR; glutathione: dehydroascorbate oxidoreductase, EC 1.8.5.1) is an enzyme that is critical for maintenance of an appropriate level of ascorbate in plant cells by the cycling of dehydroascorbate to replenish ascorbate. DHAR was considered a specific enzyme of the ascorbate-glutathione cycle. However, at least four distinct proteins can catalyze *in vitro* both glutathione-dependent DHA reduction and other reactions mainly related to thiol-disulphide exchange. These glutaredoxin enzymes (thioltransferases) have both thiol-disulfide  
25 oxidoreductase and dehydroascorbate reductase activities (Kato et al., 1997. *Plant & Cell Physiology* 38: 173-178; Detullio et al., 1998. *Plant Physiology & Biochemistry* 36: 433-440). Therefore glutaredoxins may also function *in vivo* as DHAR.

- 30 There have been no reports on the mutation or overexpression of either thioredoxin or glutaredoxin in plant cells to determine their function in terms of oxidative stress tolerance or drought tolerance.

It is the object of this invention to identify new, unique genes capable of conferring stress tolerance to plants upon over-expression.

The present invention provides a transgenic plant cell transformed by Oxidoreductase Stress-Related Protein (ORSRP) coding nucleic acid, wherein expression of the nucleic acid sequence in the plant cell results in increased tolerance and/or resistance to environmental stress as compared to a corresponding non-transformed wild type plant cell. One preferred wild type plant cell is a non-transformed Arabidopsis plant cell. An example here is the Arabidopsis wild type C24 (Nottingham Arabidopsis Stock Centre, UK ; NASC Stock N906).

Preferably the oxidoreductase stress related protein is heat-stable. The invention provides that the environmental stress can be salinity, drought, temperature, metal, chemical, pathogenic and oxidative stresses, or combinations thereof.

The object of the invention is a transgenic plant cell, wherein the ORSRP is heat-stable. Further, in said transgenic plant cell, the ORSRP is selected from yeast or plant. Preferably, in a transgenic plant of the instant invention, the ORSRP is selected from the group comprising glutaredoxin and/or thioredoxin protein.

Further the invention pertains to a transgenic plant cell, wherein the ORSRP coding nucleic acid is selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof. Object of the invention is also a transgenic plant cell, wherein the ORSRP coding nucleic acid is at least about 50 % homologous to SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49.

The invention further provides a seed produced by a transgenic plant transformed by a ORSRP coding nucleic acid, wherein the plant is true breeding for increased tolerance to environmental stress as compared to a wild type plant cell. The transgenic plant might be a monocot, a dicot or a gymnosperm plant. The invention further provides a seed produced by a transgenic plant expressing an ORSRP wherein the plant is true breeding for increased tolerance to environmental stress as compared to a wild type plant cell.

The invention further provides an agricultural product produced by any of the below-described transgenic plants, plant parts such as leafs, roots, stems, buds, flowers or seeds. The invention further provides a isolated recombinant expression vector comprising a ORSRP encoding nucleic acid.

The invention further provides a method of producing a transgenic plant with a ORSRP coding nucleic acid, wherein expression of the nucleic acid in the plant results in increased tolerance to environmental stress as compared to a wild type plant comprising: (a) transforming a plant cell with an expression vector comprising a ORSRP coding nucleic acid, and (b) generating from the plant cell a transgenic plant with an increased tolerance to environmental stress as compared to a wild type plant.

With regard to invention described here, "transgenic or transgene" means all those plants or parts thereof which have been brought about by genetic manipulation methods and in which either

- 10 a) the nucleic acid sequence as depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 or a homologue thereof, or
- b) a genetic regulatory element, for example a promoter, which is functionally linked to the nucleic acid sequence as depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 or a homologue thereof, or
- 15 c) (a) and (b)

is/are not present in its/their natural genetic environment or has/have been modified by means of genetic manipulation methods, it being possible for the modification to be, by way of example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide radicals. "Natural genetic environment" means the natural chromosomal locus in the organism of origin or the presence in a genomic library. In the case of a genomic library, the natural, genetic environment of the nucleic acid sequence is preferably at least partially still preserved. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, particularly preferably at least 1000 bp, very particularly preferably at least 5000 bp.

In said method, the used ORSRP is heat-stable. Further, the ORSRP used in the instant method described above is a glutaredoxin or thioredoxin protein. Herein the ORSRP coding nucleic acid is selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof. Further, the ORSRP coding nucleic acid used in the said method is at least about 50%

homologous to SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49.

5 A plant or plant cell is considered "true breeding" for a particular trait if it is genetically homozygous for that trait to the extent that, when the true-breeding plant is self-pollinated, a significant amount of independent segregation of the trait among the progeny is not observed. In the present invention, the trait arises from the transgenic expression of one or more DNA sequences introduced into a plant cell or plant.

10 The present invention also provides methods of modifying stress tolerance of a plant comprising, modifying the expression of a ORSRP nucleic acid in the plant. The invention provides one method of producing a transgenic plant with a synthetic, novel or modified transcription factor that acts by increasing or decreasing the transcription of a ORSRP gene.

15 The present invention also provides methods of modifying stress tolerance of a crop plant comprising utilizing a ORSRP coding nucleic acid sequence to identify individual plants in populations segregating for either increased or decreased environmental stress tolerance (DNA marker).

20 In the said method of modifying stress tolerance of a plant the ORSRP encoding nucleic acid is selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof. Further the ORSRP coding nucleic acid used therein is at least about 50% homologous to SEQ ID No. SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49. Also an expression vector as described in the instant invention might be used in the said method.

25 In an variant method of said method of modifying stress tolerance, the plant is transformed with an inducible promoter that directs expression of the ORSRP. For example, the promoter is tissue specific. In a variant method, the used promoter is developmentally regulated.

30 In the instant method of modifying stress tolerance in plant the ORSRP expression is modified by administration of an antisense molecule and/or by double stranded RNA interference that inhibits expression of ORSPR. In another variant of the method, ORSRP expression is modified by administration of an targeting nucleic sequence complementary to the regulatory region of the ORSRP encoding nucleic acid and/or by a transcription factor and/or by a zinc finger protein.

The present invention relates to a method for the identification of loci for stress tolerance phenotypes in individual plants. Genomic regions associated with environmental stress tolerance can be identified using Quantitative Trait Loci (QTL) mapping analysis. This approach may use either variation in the glutaredoxin or thioredoxin nucleic acid sequence, variation in the surrounding genomic sequences or variation in the expression level of glutaredoxin or thioredoxin nucleic acid sequence as the quantitative trait.

The invention provides that the above methods can be performed such that the stress tolerance is either increased or decreased.

This invention is not limited to specific nucleic acids, specific polypeptides, specific cell types, specific host cells, specific conditions, or specific methods, etc., as such may, of course, vary, and the numerous modifications and variations therein will be apparent to those skilled in the art. It is also to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

The present invention describes that particularly glutaredoxin or thioredoxin genes are useful for increasing a plant's tolerance and/or resistance to environmental stress. Accordingly, the present invention provides glutaredoxin and thioredoxin gene sequences selected from the group consisting of SEQ ID No. 1, 3, 5, 7, 9, 11, 13 from *Saccharomyces cerevisiae*.

This invention provides sequences of glutaredoxin and thioredoxin nucleic acids that are responsive to drought and environmental conditions in *Brassica napus*, *Arabidopsis thaliana* and *Oryza sativa* according to SEQ ID 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 and that exhibit homology at the nucleic acid and amino acid level to the yeast genes in SEQ ID 3 and 7, respectively. These plant homologs are functionally equivalent according to this invention to yeast genes of SEQ ID 3 and 7 and can be used to provide environmental stress tolerance in plants.

The invention also pertains to an isolated Oxidoreductase Stress Related Protein (ORSRP) which is selected from the group comprising SEQ ID No. 16, 18, 20, 22, 24, 44 and 50. Further the isolated Oxidoreductase Stress Related Protein (ORSRP) as mentioned before is heat-stable. The isolated Oxidoreductase Stress Related Protein (ORSRP) selected from the group comprising SEQ ID No. 16, 18, 20, 22, 24, 44 and 50 is selected from plant. Preferred is an isolated

Oxidoreductase Stress Related Protein (ORSRP) selected from the group comprising SEQ ID No. 16, 18, 20, 22, 24, 44 and 50 wherein the ORSRP is a glutaredoxin or thioredoxin protein.

5 Another object of the instant invention is an isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid selected from the group comprising SEQ ID No. 15, 17, 19, 21, 23, 45 and 49. Said isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid encoding an ORSRP which is heat-stable. Thereby the isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid selected from the group comprising SEQ ID No. 15, 17, 19, 21, 23, 45 and 49 encoding an ORSRP which is selected from plants. Preferred is an  
10 isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid selected from the group comprising SEQ ID No. 15, 17, 19, 21, 23, 45 and 49 wherein the ORSRP is a glutaredoxin or thioredoxin .

Homologs of the aforementioned sequences can be isolated advantageously from yeast,  
15 fungi or plants, preferably from yeasts such as from the genera *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Torulopsis* or *Schizosaccharomyces*, or plants such as *Arabidopsis thaliana*, maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, borage, sunflower, linseed, primrose, rapeseed, canola and turnip rape, manihot, pepper, sunflower, tagetes, solanaceous plant such as potato, tobacco, eggplant and tomato, *Vicia* species, pea, alfalfa, bushy plants such  
20 as coffee, cacao, tea, *Salix* species, trees such as oil palm, coconut, perennial grass, such as ryegrass and fescue, and forage crops, such as alfalfa and clover and from spruce, pine or fir for example, more preferably from *Saccharomyces cerevisiae* or plants.

The glutaredoxin or thioredoxin of the present invention are preferably produced by  
25 recombinant DNA techniques. For example, a nucleic acid molecule encoding the protein is cloned into an expression vector, for example in to a binary vector, the expression vector is introduced into a host cell, for example the *Arabidopsis thaliana* wild type NASC N906 or any other plant cell as described in the examples see below, and the glutaredoxin or thioredoxin is expressed in said host cell. Examples for binary vectors are pBIN19, pBI101, pBinAR, pGPTV  
30 or pPZP (Hajukiewicz, P. et al., 1994, *plant Mol. Biol.*, 25: 989-994).

As used herein, the term "environmental stress" refers to any sub-optimal growing condition and includes, but is not limited to, sub-optimal conditions associated with salinity, drought, temperature, metal, chemical, pathogenic and oxidative stresses, or combinations thereof. In preferred embodiments, the environmental stress can be salinity, drought, heat, or low temperature, or combinations thereof, and in particular, can be low water content or low temperature. Wherein drought stress means any environmental stress which leads to a lack of water in plants or reduction of water supply to plants, wherein low temperature stress means freezing of plants below + 4 °C as well as chilling of plants below 15 °C and wherein high temperature stress means for example a temperature above 35 °C. The range of stress and stress response depends on the different plants which are used for the invention, i.e. it differs for example between a plant such as wheat and a plant such as Arabidopsis. It is also to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized.

As also used herein, the terms "nucleic acid" and "nucleic acid molecule" are intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. This term also encompasses untranslated sequence located at both the 3' and 5' ends of the coding region of the gene: at least about 1000 nucleotides of sequence upstream from the 5' end of the coding region and at least about 200 nucleotides of sequence downstream from the 3' end of the coding region of the gene. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one that is substantially separated from other nucleic acid molecules, which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of some of the sequences that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated glutaredoxin or thioredoxin nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be free from some of the other cellular



material with which it is naturally associated, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule encoding an ORSRP or a portion thereof which confers tolerance and/or resistance to environmental stress in plants, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, a *Arabidopsis thaliana* glutaredoxin or thioredoxin cDNA can be isolated from a *A. thaliana* library using all or portion of one of the sequences of SEQ ID 1, 3, 5, 7, 9, 11, 13 of yeast. Moreover, a nucleic acid molecule encompassing all or a portion of one of the sequences of SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this sequence. For example, mRNA can be isolated from plant cells (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al., 1979 Biochemistry 18:5294-5299) and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon one of the nucleotide sequences shown in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast. A nucleic acid molecule of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecule so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to a glutaredoxin or thioredoxin nucleotide sequence can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises one of the nucleotide sequences shown in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants encoding the glutaredoxin or thioredoxin (i.e., the "coding region"), as well as 5' untranslated sequences and 3' untranslated sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the coding region of one of the sequences in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants, for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a glutaredoxin or thioredoxin.

Portions of proteins encoded by the glutaredoxin or thioredoxin nucleic acid molecules of the invention are preferably biologically active portions of one of the glutaredoxin or thioredoxin described herein. As used herein, the term "biologically active portion of" a glutaredoxin or thioredoxin is intended to include a portion, e.g., a domain/motif, of a glutaredoxin or thioredoxin that participates in a stress tolerance and/or resistance response in a plant. To determine whether a glutaredoxin or thioredoxin, or a biologically active portion thereof, results in increased stress tolerance in a plant, a stress analysis of a plant comprising the glutaredoxin or thioredoxin may be performed. Such analysis methods are well known to those skilled in the art, as detailed in the Examples. More specifically, nucleic acid fragments encoding biologically active portions of a glutaredoxin or thioredoxin can be prepared by isolating a portion of one of the sequences in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants, expressing the encoded portion of the glutaredoxin or thioredoxin or peptide (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the glutaredoxin or thioredoxin or peptide.

Biologically active portions of a glutaredoxin or thioredoxin are encompassed by the present invention and include peptides comprising amino acid sequences derived from the amino acid sequence of a glutaredoxin or thioredoxin gene, or the amino acid sequence of a protein homologous to a glutaredoxin or thioredoxin, which include fewer amino acids than a full length glutaredoxin or thioredoxin or the full length protein which is homologous to a glutaredoxin or thioredoxin, and exhibits at least some enzymatic activity of a glutaredoxin or thioredoxin. Typically, biologically active portions (e.g., peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) comprise a domain or motif with at least one activity of a glutaredoxin or thioredoxin enzyme. Moreover, other biologically active portions in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein. Preferably, the biologically active portions of a glutaredoxin or thioredoxin include one or more selected domains/motifs or portions thereof having biological activity.

In addition to fragments of the glutaredoxin or thioredoxin described herein, the present invention includes homologs and analogs of naturally occurring glutaredoxin or thioredoxin and glutaredoxin or thioredoxin encoding nucleic acids in a plant.

"Homologs" are defined herein as two nucleic acids or proteins that have similar, or "homologous", nucleotide or amino acid sequences, respectively. Homologs include allelic variants, orthologs, paralogs, agonists and antagonists of glutaredoxin or thioredoxin as defined

hereafter. The term "homolog" further encompasses nucleic acid molecules that differ from one of the nucleotide sequences shown in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants (and portions thereof) due to degeneracy of the genetic code and thus encode the same glutaredoxin or thioredoxin as that encoded by the amino acid sequences shown in SEQ ID No. 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants. As used herein a "naturally occurring" glutaredoxin or thioredoxin refers to a glutaredoxin or thioredoxin amino acid sequence that occurs in nature.

Moreover, nucleic acid molecules encoding glutaredoxin or thioredoxin from the same or other species such as glutaredoxin or thioredoxin analogs, orthologs and paralogs, are intended to be within the scope of the present invention. As used herein, the term "analogs" refers to two nucleic acids that have the same or similar function, but that have evolved separately in unrelated organisms. As used herein, the term "orthologs" refers to two nucleic acids from different species that have evolved from a common ancestral gene by speciation. Normally, orthologs encode proteins having the same or similar functions. As also used herein, the term "paralogs" refers to two nucleic acids that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related (Tatusov, R.L. et al. 1997 Science 278(5338):631-637). Analogs, orthologs and paralogs of a naturally occurring glutaredoxin or thioredoxin can differ from the naturally occurring glutaredoxin or thioredoxin by post-translational modifications, by amino acid sequence differences, or by both. Post-translational modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation, and such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. In particular, orthologs of the invention will generally exhibit at least 80-85%, more preferably 90%, and most preferably 95%, 96%, 97%, 98% or even 99% identity or homology with all or part of a naturally occurring glutaredoxin or thioredoxin amino acid sequence and will exhibit a function similar to a glutaredoxin or thioredoxin. Orthologs of the present invention are also preferably capable of participating in the stress response in plants.

In addition to naturally-occurring variants of a glutaredoxin or thioredoxin sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into a nucleotide sequence of SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants, thereby leading to changes in the amino acid sequence of the encoded glutaredoxin or thioredoxin,

without altering the functional ability of the glutaredoxin or thioredoxin . For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a sequence of SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of one of the glutaredoxin or thioredoxin s without altering the activity of said glutaredoxin or thioredoxin , whereas an "essential" amino acid residue is required for glutaredoxin or thioredoxin activity. Other amino acid residues, however, (e.g., those that are not conserved or only semi-conserved in the domain having glutaredoxin or thioredoxin activity) may not be essential for activity and thus are likely to be amenable to alteration without altering glutaredoxin or thioredoxin activity.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding glutaredoxin or thioredoxin that contain changes in amino acid residues that are not essential for glutaredoxin or thioredoxin activity. Such glutaredoxin or thioredoxin differ in amino acid sequence from a sequence comprising of SEQ IDs 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants, yet retain at least one of the glutaredoxin or thioredoxin activities described herein. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50% homologous to an amino acid sequence of SEQ IDs 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50. Preferably, the protein encoded by the nucleic acid molecule is at least about 50-60% homologous to one of the sequences of SEQ ID No. 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants, more preferably at least about 60-70% homologous to one of the sequences of SEQ ID No. 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants, even more preferably at least about 70-80%, 80-90%, 90-95% homologous to one of the sequences of SEQ ID No. 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants, and most preferably at least about 96%, 97%, 98%, or 99% homologous to one of the sequences of SEQ IDs 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants. The preferred glutaredoxin or thioredoxin homologs of the present invention are preferably capable of participating in the stress tolerance response in a plant. The homology (= identity) was calculated over the entire amino acid range. The program used was PileUp (J. Mol. Evolution., 25 (1987), 351-360, Higgins et al., CABIOS, 5 1989: 151-153).

Variants shall also be encompassed, in particular, functional variants which can be obtained from the sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants by means of deletion, insertion or substitution of nucleotides, the enzymatic activity of the derived synthetic proteins being retained.

An isolated nucleic acid molecule encoding a glutaredoxin or thioredoxin homologous to a protein sequence of SEQ IDs 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into one of the sequences of SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a glutaredoxin or thioredoxin is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a glutaredoxin or thioredoxin coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for a glutaredoxin or thioredoxin activity described herein to identify mutants that retain glutaredoxin or thioredoxin activity. Following mutagenesis of one of the sequences of SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants, the encoded protein can be expressed

recombinantly and the activity of the protein can be determined by analyzing the stress tolerance of a plant expressing the protein as described in Examples below.

In addition to the nucleic acid molecules encoding the glutaredoxin or thioredoxin described above, another aspect of the invention pertains to isolated nucleic acid molecules that are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire glutaredoxin or thioredoxin coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a glutaredoxin or thioredoxin. The term "coding region" refers to the region of the nucleotide sequence comprising codons that are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a glutaredoxin or thioredoxin. The term "noncoding region" refers to 5' and 3' sequences that flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of one of the nucleotide sequences shown in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants, or a portion thereof. A nucleic acid molecule that is complementary to one of the nucleotide sequences shown in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants is one which is sufficiently complementary to one of the nucleotide sequences shown in SEQ IDs 3 or 7 such that it can hybridize to one of these nucleotide sequences, thereby forming a stable duplex.

Given the coding strand sequences encoding the glutaredoxin or thioredoxin disclosed herein (e.g., the sequences set forth in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of glutaredoxin or thioredoxin mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of glutaredoxin or thioredoxin mRNA. For

example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of glutaredoxin or thioredoxin mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 or more nucleotides in length.

It is also possible to use the inverted repeat technology combining an antisense fragment with a portion of the antisense fragment in sense orientation linked by either an adapter sequence or an excisable intron (Abstract Book of the 6th Intern. Congr. Of Plant Mol. Biol. ISPMB, Quebec June 18-24,2000, Abstract No. S20-9 by Green et al.).

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a glutaredoxin or thioredoxin to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide

complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong prokaryotic, viral, or eukaryotic (including plant) promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier et al., 1987 Nucleic Acids. Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., 1987 Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al., 1987 FEBS Lett. 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes described in Haselhoff and Gerlach, 1988 Nature 334:585-591) can be used to catalytically cleave glutaredoxin or thioredoxin mRNA transcripts to thereby inhibit translation of glutaredoxin or thioredoxin mRNA. A ribozyme having specificity for a glutaredoxin or thioredoxin -encoding nucleic acid can be designed based upon the nucleotide sequence of a glutaredoxin or thioredoxin cDNA, as disclosed herein (i.e., SEQ IDs 1-76) or on the basis of a heterologous sequence to be isolated according to methods taught in this invention. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a glutaredoxin or thioredoxin -encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071 and Cech et al. U.S. Patent No. 5,116,742. Alternatively, glutaredoxin or thioredoxin mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W., 1993 Science 261:1411-1418.



Another embodiment of the invention is the regulating of the glutaredoxin or thioredoxin genes by means of double-stranded RNA ("double-stranded RNA interference"; dsRNAi) which has been described repeatedly for animal and plant organisms (for example Matzke MA et al. (2000) Plant Mol Biol 43:401-415; Fire A. et al (1998) Nature 391:806-811; WO 99/32619; WO 99/53050; WO 00/68374; WO 00/44914; WO 00/44895; WO 00/49035; WO 00/63364). Express  
5 reference is made to the processes and methods described in the above references. Such effective gene suppression can for example also be demonstrated upon transient expression or following transient transformation for example as the consequence of biolistic transformation (Schweizer P et al. (2000) Plant J 2000 24: 895-903). dsRNAi methods are based on the phenomenon that the  
10 simultaneous introduction of complementary strand and counterstrand of a gene transcript causes the expression of the gene in question to be suppressed in a highly efficient manner. The phenotype caused greatly resembles a corresponding knock-out mutant (Waterhouse PM et al. (1998) Proc Natl Acad Sci USA 95:13959-64).

As described, inter alia, in WO 99/32619, dsRNAi approaches are markedly superior to  
15 traditional antisense approaches.

The invention therefore furthermore relates to double-stranded RNA molecules (dsRNA molecules) which, upon introduction into a plant (or a cell, tissue, organ or seed derived therefrom), bring about the reduction of an glutaredoxin or thioredoxin gene. In the double-stranded RNA molecule for reducing the expression of an glutaredoxin or thioredoxin protein,

- 20 a) one of the two RNA strands is essentially identical to at least a portion of an glutaredoxin or thioredoxin nucleic acid sequence, and
- b) the corresponding other RNA strand is essentially identical to at least a portion of the complementary strand of an glutaredoxin or thioredoxin nucleic acid sequence.

25 "Essentially identical" means that the dsRNA sequence can also show insertions, deletions or individual point mutations compared with the glutaredoxin or thioredoxin target sequence while still bringing about an effective reduction of the expression. The homology in accordance with the above definition preferably amounts to at least 75%, preferably at least 80%, very especially preferably at least 90%, most preferably 100%, between the sense strand of an  
30 inhibitory dsRNA and a part-segment of an glutaredoxin or thioredoxin nucleic acid sequence (or between the antisense strand and the complementary strand of an glutaredoxin or thioredoxin nucleic acid sequence). The length of the part-segment amounts to at least 10 bases, preferably at least 25 bases, especially preferably at least 50 bases, very especially preferably at least 100

bases, most preferably at least 200 bases or at least 300 bases. As an alternative, an "essentially identical" dsRNA can also be defined as a nucleic acid sequence which is capable of hybridizing with part of an glutaredoxin or thioredoxin gene transcript (for example in 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA at 505C or 705C for 12 to 16 h).

- 5           The dsRNA can be composed of one or more strands of polymerized ribonucleotides. Modifications both of the sugar-phosphate backbone and of the nucleosides may be present. For example, the phosphodiester bonds of the natural RNA can be modified in such a way that they comprise at least one nitrogen or sulfur hetero atom. Bases can be modified in such a way that the activity of, for example, adenosine deaminase is restricted.

10

The dsRNA can be generated enzymatically or fully or partially synthesized chemically.

- The double-stranded structure can be formed starting from an individual self-complementary strand or starting from two complementary strands. In a single self-complementary strand, sense and antisense sequence may be linked by a linking sequence ("linker") and can form for example a hairpin structure. The linking sequence can preferably be an intron which is spliced out after the dsRNA has been synthesized. The nucleic acid sequence encoding a dsRNA can comprise further elements such as, for example, transcription termination signals or polyadenylation signals. If the two dsRNA strands are to be combined in a cell or plant, this can be effected in various ways:

- 20           a) transformation of the cell or plant with a vector comprising both expression cassettes,
- b) cotransformation of the cell or plant with two vectors, one of them comprising the expression cassettes with the sense strand and the other comprising the expression cassettes with the antisense strand,
- 25           c) hybridizing two plants, each of which has been transformed with one vector, one of the vectors comprising the expression cassettes with the sense strand and the other comprising the expression cassettes with the antisense strand.

- The formation of the RNA duplex can be initiated either outside or within the cell. Like in WO 99/53050, the dsRNA can also encompass a hairpin structure by linking sense and antisense strand by means of a linker (for example an intron). The self-complementary dsRNA structures are preferred since they only require the expression of one construct and always comprise the complementary strands in an equimolar ratio.
- 30

The expression cassettes encoding the antisense or sense strand of a dsRNA or the self-complementary strand of the dsRNA are preferably inserted into a vector and, using the methods described herein, stably inserted into the genome of a plant in order to ensure permanent expression of the dsRNA, using selection markers for example.

5 The dsRNA can be introduced using a quantity which allows at least one copy per cell. Greater quantities (for example at least 5, 10, 100, 500 or 1000 copies per cell) may bring about a more effective reduction.

10 As already described, 100% sequence identity between dsRNA and an glutaredoxin or thioredoxin gene transcript is not necessarily required in order to bring about an effective reduction of the glutaredoxin or thioredoxin expression. Accordingly, there is the advantage that the method is tolerant with regard to sequence deviations as may exist as the consequence of genetic mutations, polymorphisms or evolutionary divergence. Thus, for example, it is possible to use the dsRNA generated on the basis of the glutaredoxin or thioredoxin sequence of one organism to suppress the glutaredoxin or thioredoxin expression in another organism. The high  
15 sequence homology between the glutaredoxin or thioredoxin sequences from different sources allows the conclusion that this protein is conserved to a high degree within plants, so that the expression of a dsRNA derived from one of the disclosed glutaredoxin or thioredoxin sequences as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 of yeast and/or SEQ ID No. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50 of plants appears to have an advantageous effect in  
20 other plant species as well.

The dsRNA can be synthesized either in vivo or in vitro. To this end, a DNA sequence encoding a dsRNA can be brought into an expression cassette under the control of at least one genetic control element (such as, for example, promoter, enhancer, silencer, splice donor or splice acceptor or polyadenylation signal). Suitable advantageous constructions are described herein.  
25 Polyadenylation is not necessarily required, nor do elements for initiating translation have to be present.

A dsRNA can be synthesized chemically or enzymatically. Cellular RNA polymerases or bacteriophage RNA polymerases (such as, for example, T3, T7 or SP6 RNA polymerase) can be used for this purpose. Suitable methods for expression of RNA in vitro are described (WO  
30 97/32016; US 5,593,874; US 5,698,425, US 5,712,135, US 5,789,214, US 5,804,693). A dsRNA which has been synthesized in vitro chemically or enzymatically can be isolated completely or to some degree from the reaction mixture, for example by extraction, precipitation, electrophoresis, chromatography or combinations of these methods, before being introduced into a cell, tissue or

organism. The dsRNA can be introduced directly into the cell or else be applied extracellularly (for example into the interstitial space).

However, it is preferred to transform the plant stably with an expression construct which brings about the expression of the dsRNA. Suitable methods are described herein. The methods of dsRNAi, cosuppression by means of sense RNA and "VIGS" ("virus induced gene silencing") are also termed "post-transcriptional gene silencing" (PTGS). PTGS methods, like the reduction of the glutaredoxin or thioredoxin function or activity with dominant-negative glutaredoxin or thioredoxin variants, are especially advantageous because the demands regarding the homology between the endogenous gene to be suppressed and the sense or dsRNA nucleic acid sequence expressed recombinantly (or between the endogenous gene and its dominant-negative variant) are lower than, for example, in the case of a traditional antisense approach. Such criteria with regard to homology are mentioned in the description of the dsRNAi method and can generally be applied to PTGS methods or dominant-negative approaches. Owing to the high degree of homology between the glutaredoxin or thioredoxin proteins from different sources, a high degree of conservation of this protein in plants can be assumed. Thus, using the glutaredoxin or thioredoxin nucleic acid sequences from yeast, it is presumably also possible efficiently to suppress the expression of homologous glutaredoxin or thioredoxin proteins in other species such as plants without the isolation and structure elucidation of the glutaredoxin or thioredoxin homologs occurring therein being required. Considerably less labor is therefore required.

All of the substances and compounds which directly or indirectly bring about a reduction in protein quantity, RNA quantity, gene activity or protein activity of an glutaredoxin or thioredoxin protein shall subsequently be combined in the term "anti- glutaredoxin or thioredoxin " compounds. The term "anti- glutaredoxin or thioredoxin " compound explicitly includes the nucleic acid sequences, peptides, proteins or other factors employed in the above-described methods.

For the purposes of the invention, "introduction" comprises all of the methods which are capable of directly or indirectly introducing an "anti- glutaredoxin or thioredoxin " compound into a plant or a cell, compartment, tissue, organ or seed thereof, or of generating such a compound there. Direct and indirect methods are encompassed. The introduction can lead to a transient presence of an "anti- glutaredoxin or thioredoxin " compound (for example a dsRNA) or else to its stable presence.

Alternatively, glutaredoxin or thioredoxin gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a glutaredoxin or thioredoxin

nucleotide sequence (e.g., a glutaredoxin or thioredoxin promoter and/or enhancer) to form triple helical structures that prevent transcription of a glutaredoxin or thioredoxin gene in target cells. See generally, Helene, C., 1991 *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al., 1992 *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J., 1992 *Bioassays* 14(12):807-15.

5 In particular, a useful method to ascertain the level of transcription of the gene (an indicator of the amount of mRNA available for translation to the gene product) is to perform a Northern blot (for reference see, for example, Ausubel et al., 1988 *Current Protocols in Molecular Biology*, Wiley: New York). This information at least partially demonstrates the degree of transcription of the transformed gene. Total cellular RNA can be prepared from cells, tissues or  
10 organs by several methods, all well-known in the art, such as that described in Bormann, E.R. et al., 1992 *Mol. Microbiol.* 6:317-326. To assess the presence or relative quantity of protein translated from this mRNA, standard techniques, such as a Western blot, may be employed. These techniques are well known to one of ordinary skill in the art. (See, for example, Ausubel et al., 1988 *Current Protocols in Molecular Biology*, Wiley: New York).

15 The invention further provides an isolated recombinant expression vector comprising a Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin nucleic acid as described above, wherein expression of the vector or glutaredoxin or thioredoxin nucleic acid, respectively in a host cell results in increased tolerance and/or resistance to environmental stress  
20 as compared to the wild type of the host cell. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of  
25 autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such  
30 vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression

vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells and operably linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from *Agrobacterium tumefaciens* T-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984 EMBO J. 3:835) or functional equivalents thereof but also all other terminators functionally active in plants are suitable.

As plant gene expression is very often not limited on transcriptional levels, a plant expression cassette preferably contains other operably linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco mosaic virus enhancing the protein per RNA ratio (Gallie et al., 1987 Nucl. Acids Research 15:8693-8711).

Plant gene expression has to be operably linked to an appropriate promoter conferring gene expression in a timely, cell or tissue specific manner. Preferred are promoters driving constitutive expression (Benfey et al., 1989 EMBO J. 8:2195-2202) like those derived from plant viruses like the 35S CaMV (Franck et al., 1980 Cell 21:285-294), the 19S CaMV (see also U.S. Patent No. 5352605 and PCT Application No. WO 8402913) or plant promoters like those from Rubisco small subunit described in U.S. Patent No. 4,962,028.

Additional advantageous regulatory sequences are, for example, included in the plant promoters such as CaMV/35S [Franck et al., Cell 21 (1980) 285 - 294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, LEB4, nos or in the ubiquitin, napin or phaseolin promoter. Also advantageous in this connection are inducible promoters such as the promoters described in EP-A-0 388 186 (benzyl sulfonamide inducible), Plant J. 2, 1992: 397 - 404 (Gatz et al., Tetracyclin inducible), EP-A-0 335 528 (abscisic acid inducible) or WO 93/21334 (ethanol or cyclohexenol inducible). Additional useful plant promoters are the cytosolic FBPase promotor or ST-LSI promoter of the potato (Stockhaus et al., EMBO J. 8, 1989, 2445), the phosphorybosyl phyrophoshate amido transferase promoter of Glycine max (gene bank accession No. U87999) or the noden specific promoter described in EP-A-0 249 676. Additional particularly advantageous promoters are seed specific promoters which can be used for monokotyledones or dikotyledones are described in US 5,608,152 (napin promoter from rapeseed), WO 98/45461 (phaseolin promoter from Arabidopsis), US 5,504,200 (phaseolin

promoter from *Phaseolus vulgaris*), WO 91/13980 (Bce4 promoter from Brassica), Baeumlein et al., Plant J., 2, 2, 1992: 233-239 (LEB4 promoter from leguminosa) said promoters are useful in dikotyledones. The following promoters are useful for example in monokotyledones lpt-2- or lpt-1- promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other useful promoters described in WO 99/16890.

It is possible in principle to use all natural promoters with their regulatory sequences like those mentioned above for the novel process. It is also possible and advantageous in addition to use synthetic promoters.

The gene construct may also comprise further genes which are to be inserted into the organisms and which are for example involved in stress resistance. It is possible and advantageous to insert and express in host organisms regulatory genes such as genes for inducers, repressors or enzymes which intervene by their enzymatic activity in the regulation, or one or more or all genes of a biosynthetic pathway. These genes can be heterologous or homologous in origin. The inserted genes may have their own promoter or else be under the control of same promoter as the sequences SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants or their homologs.

The gene construct advantageously comprises, for expression of the other genes present, additionally 3' and/or 5' terminal regulatory sequences to enhance expression, which are selected for optimal expression depending on the selected host organism and gene or genes.

These regulatory sequences are intended to make specific expression of the genes and protein expression possible as mentioned above. This may mean, depending on the host organism, for example that the gene is expressed or overexpressed only after induction, or that it is immediately expressed and/or overexpressed.

The regulatory sequences or factors may moreover preferably have a beneficial effect on expression of the introduced genes, and thus increase it. It is possible in this way for the regulatory elements to be enhanced advantageously at the transcription level by using strong transcription signals such as promoters and/or enhancers. However, in addition, it is also possible to enhance translation by, for example, improving the stability of the mRNA.

Other preferred sequences for use in plant gene expression cassettes are targeting-sequences necessary to direct the gene product in its appropriate cell compartment (for review see Kermode, 1996 Crit. Rev. Plant Sci. 15(4):285-423 and references cited therein) such as the vacuole, the nucleus, all types of plastids like amyloplasts, chloroplasts, chromoplasts, the

extracellular space, mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells.

Plant gene expression can also be facilitated via an inducible promoter (for review see Gatz, 1997 Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if gene expression is wanted to occur in a time specific manner.

Table 1 lists several examples of promoters that may be used to regulate transcription of the glutaredoxin or thioredoxin nucleic acid coding sequences.

Tab. 1: Examples of Tissue-specific and Stress inducible promoters in plants

Expression	Reference
Cor78- Cold, drought, salt, ABA, wounding-inducible	Ishitani, <i>et al.</i> , Plant Cell 9:1935-1949 (1997). Yamaguchi-Shinozaki and Shinozaki, Plant Cell 6:251-264 (1994).
Rci2A - Cold, dehydration-inducible	Capel <i>et al.</i> , Plant Physiol 115:569-576 (1997)
Rd22 - Drought, salt	Yamaguchi-Shinozaki and Shinozaki, Mol Gen Genet 238:17-25 (1993).
Cor15A - Cold, dehydration, ABA	Baker <i>et al.</i> , Plant Mol. Biol. 24:701-713 (1994).
GH3- Auxin inducible	Liu <i>et al.</i> , Plant Cell 6:645-657 (1994)
ARSK1-Root, salt inducible	Hwang and Goodman, Plant J 8:37-43 (1995).
PtxA - Root, salt inducible	GenBank accession X67427
SbHRGP3 - Root specific	Ahn <i>et al.</i> , Plant Cell 8:1477-1490 (1998).
KST1 - Guard cell specific	Plesch <i>et al.</i> , unpublished manuscript; Müller-Röber <i>et al.</i> , EMBO J. 14:2409-2416 (1995).
KAT1 - Guard cell specific	Plesch <i>et al.</i> , Gene 249:83-89 (2000) Nakamura <i>et al.</i> , Plant Physiol. 109:371-374 (1995)
salicylic acid inducible	PCT Application No. WO 95/19443
tetracycline inducible	Gatz <i>et al.</i> Plant J. 2:397-404 (1992)
Ethanol inducible	PCT Application No. WO 93/21334
pathogen inducible PRP1	Ward <i>et al.</i> , 1993 Plant. Mol. Biol. 22:361-366
heat inducible hsp80	U.S. Patent No. 5187267
cold inducible alpha-amylase	PCT Application No. WO 96/12814
Wound-inducible pinII	European Patent No. 375091
RD29A - salt-inducible	Yamaguchi-Shinozaki <i>et al.</i> (1993) Mol. Gen. Genet. 236:331-340
plastid-specific viral RNA-polymerase	PCT Application No. WO 95/16783 and. WO 97/06250



Other selection marker systems, like the AHAS marker or other promoters, e.g. superpromotor (Ni et al., Plant Journal 7, 1995: 661-676), Ubiquitin promotor (Callis et al., J. Biol. Chem., 1990, 265: 12486-12493; US 5,510,474; US 6,020,190; Kawalleck et al., Plant Molecular Biology, 1993, 21: 673-684) or 34S promotor (GenBank Accession numbers M59930 and X16673) were similar useful for the instant invention and are known to a person skilled in the art.

The invention further provides a recombinant expression vector comprising a glutaredoxin or thioredoxin DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to a glutaredoxin or thioredoxin mRNA. Regulatory sequences operatively linked to a nucleic acid molecule cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types. For instance, viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus wherein antisense nucleic acids are produced under the control of a high efficiency regulatory region. The activity of the regulatory region can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics, Vol. 1(1) 1986 and Mol et al., 1990 FEBS Letters 268:427-430.

Gene expression in plants is regulated by the interaction of protein transcription factors with specific nucleotide sequences within the regulatory region of a gene. A common type of transcription factor contains zinc finger (ZF) motifs. Each ZF module is approximately 30 amino acids long folded around a zinc ion. The DNA recognition domain of a ZF protein is a  $\alpha$ -helical structure that inserts into the major groove of the DNA double helix. The module contains three amino acids that bind to the DNA with each amino acid contacting a single base pair in the target DNA sequence. ZF motifs are arranged in a modular repeating fashion to form a set of fingers that recognize a contiguous DNA sequence. For example, a three-fingered ZF motif will recognize 9 bp of DNA. Hundreds of proteins have been shown to contain ZF motifs with between 2 and 37 ZF modules in each protein (Isalan M, et al., 1998 Biochemistry 37(35):12026-33; Moore M, et al., 2001 Proc. Natl. Acad. Sci. USA 98(4):1432-1436 and 1437-1441; US patents US 6007988 and US 6013453).

The regulatory region of a plant gene contains many short DNA sequences (cis-acting elements) that serve as recognition domains for transcription factors, including ZF proteins. Similar recognition domains in different genes allow the coordinate expression of several genes encoding enzymes in a metabolic pathway by common transcription factors. Variation in the recognition domains among members of a gene family facilitates differences in gene expression within the same gene family, for example, among tissues and stages of development and in response to environmental conditions.

Typical ZF proteins contain not only a DNA recognition domain but also a functional domain that enables the ZF protein to activate or repress transcription of a specific gene.

Experimentally, an activation domain has been used to activate transcription of the target gene (US patent 5789538 and patent application WO9519431), but it is also possible to link a transcription repressor domain to the ZF and thereby inhibit transcription (patent applications WO00/47754 and WO2001002019). It has been reported that an enzymatic function such as nucleic acid cleavage can be linked to the ZF (patent application WO00/20622)

The invention provides a method that allows one skilled in the art to isolate the regulatory region of one or more Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin genes from the genome of a plant cell and to design zinc finger transcription factors linked to a functional domain that will interact with the regulatory region of the gene. The interaction of the zinc finger protein with the plant gene can be designed in such a manner as to alter expression of the gene and thereby confer increased or decreased tolerance of abiotic stress such as drought. The invention provides a method of producing a transgenic plant with a transgene encoding this designed transcription factor, or alternatively a natural transcription factor, that modifies transcription of the Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin gene to provide increased tolerance of environmental stress.

In particular, the invention provides a method of producing a transgenic plant with a Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin coding nucleic acid, wherein expression of the nucleic acid(s) in the plant results in increased tolerance to environmental stress as compared to a wild type plant comprising: (a) transforming a plant cell with an expression vector comprising a glutaredoxin or thioredoxin nucleic acid, and (b) generating from the plant cell a transgenic plant with an increased tolerance to environmental stress as compared to a wild type plant. For such plant transformation, binary vectors such as pBinAR can be used (Höfgen and Willmitzer, 1990 Plant Science 66:221-230). Moreover suitable

binary vectors such as pBIN19, pBI101, pGPTV or pCambia are described in Hellens et al., Trends in Plant Science, 2000, 5: 446-451.

Construction of the binary vectors can be performed by ligation of the cDNA in sense or antisense orientation into the T-DNA. 5-prime to the cDNA a plant promoter activates transcription of the cDNA. A polyadenylation sequence is located 3-prime to the cDNA. Tissue-specific expression can be achieved by using a tissue specific promoter as listed above. Also, any other promoter element can be used. For constitutive expression within the whole plant, the CaMV 35S promoter can be used. The expressed protein can be targeted to a cellular compartment using a signal peptide, for example for plastids, mitochondria or endoplasmic reticulum (Kermode, 1996 Crit. Rev. Plant Sci. 4(15):285-423). The signal peptide is cloned 5-prime in frame to the cDNA to achieve subcellular localization of the fusion protein. Additionally, promoters that are responsive to abiotic stresses can be used with, such as the *Arabidopsis* promoter RD29A. One skilled in the art will recognize that the promoter used should be operatively linked to the nucleic acid such that the promoter causes transcription of the nucleic acid which results in the synthesis of a mRNA which encodes a polypeptide. Alternatively, the RNA can be an antisense RNA for use in affecting subsequent expression of the same or another gene or genes.

Alternate methods of transfection include the direct transfer of DNA into developing flowers via electroporation or *Agrobacterium* mediated gene transfer. *Agrobacterium* mediated plant transformation can be performed using for example the GV3101(pMP90) (Koncz and Schell, 1986 Mol. Gen. Genet. 204:383-396) or LBA4404 (Ooms et al., Plasmid, 1982, 7: 15-29; Hoekema et al., Nature, 1983, 303: 179-180) *Agrobacterium tumefaciens* strain. Transformation can be performed by standard transformation and regeneration techniques (Deblaere et al., 1994 Nucl. Acids. Res. 13:4777-4788; Gelvin and Schilperoort, Plant Molecular Biology Manual, 2<sup>nd</sup> Ed. - Dordrecht : Kluwer Academic Publ., 1995. - in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, B R and Thompson, J E, Methods in Plant Molecular Biology and Biotechnology, Boca Raton : CRC Press, 1993. - 360 S., ISBN 0-8493-5164-2). For example, rapeseed can be transformed via cotyledon or hypocotyl transformation (Moloney et al., 1989 Plant Cell Reports 8:238-242; De Block et al., 1989 Plant Physiol. 91:694-701). Use of antibiotics for *Agrobacterium* and plant selection depends on the binary vector and the *Agrobacterium* strain used for transformation. Rapeseed selection is normally performed using kanamycin as selectable plant marker. *Agrobacterium* mediated gene transfer to flax can be performed using, for example, a technique described by Mlynarova et al., 1994 Plant Cell Report

13:282-285. Additionally, transformation of soybean can be performed using for example a technique described in European Patent No. 0424 047, U.S. Patent No. 5,322,783, European Patent No. 0397 687, U.S. Patent No. 5,376,543 or U.S. Patent No. 5,169,770. Transformation of maize can be achieved by particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbide fiber technique. (See, for example, Freeling and Walbot "The maize handbook" Springer Verlag: New York (1993) ISBN 3-540-97826-7). A specific example of maize transformation is found in U.S. Patent No. 5,990,387 and a specific example of wheat transformation can be found in PCT Application No. WO 93/07256.

The Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin nucleic acid molecules of the invention have a variety of uses. Most importantly, the nucleic acid and amino acid sequences of the present invention can be used to transform plant cells or plants, thereby inducing tolerance to stresses such as drought, high salinity and cold. The present invention therefore provides a transgenic plant transformed by a Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin nucleic acid (coding or antisense), wherein expression of the nucleic acid sequence in the plant results in increased tolerance to environmental stress as compared to a wild type plant. The transgenic plant can be a monocot or a dicot or a gymnosperm plant. The invention further provides that the transgenic plant can be selected from maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, borage, sunflower, linseed, primrose, rapeseed, canola and turnip rape, manihot, pepper, tagetes, solanaceous plant such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa, bushy plants such as coffee, cacao, tea, Salix species, trees such as oil palm, coconut, perennial grass, such as ryegrass and fescue, and forage crops, such as alfalfa and clover and *Arabidopsis thaliana*. Further the transgenic plant can be selected from spruce, pine or fir for example.

In particular, the present invention describes using the expression of Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin to engineer drought-tolerant, salt-tolerant and/or cold-tolerant plants. This strategy has herein been demonstrated for *Arabidopsis thaliana*, Ryegrass, Alfalfa, Rapeseed/Canola, Soybean, Corn and Wheat but its application is not restricted to these plants. Accordingly, the invention provides a transgenic plant containing a glutaredoxin or thioredoxin selected from SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants, wherein the environmental stress is drought, increased salt or decreased or increased temperature but its application is not restricted to these adverse environments. Protection against other adverse

conditions such as heat, air pollution, heavy metals and chemical toxicants, for example, may be obtained. In preferred embodiments, the environmental stress is drought.

5 The present invention also provides methods of modifying stress tolerance of a plant comprising, modifying the expression of a Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin in the plant. The invention provides that this method can be performed such that the stress tolerance is either increased or decreased. This can for example be done by the use of transcription factor or some type of site specific mutagenesis agent. In particular, the present invention provides methods of producing a transgenic plant having an increased tolerance to environmental stress as compared to a wild type plant comprising  
10 increasing expression of a Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin in a plant.

The Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin encoding nucleic acids of the present invention have utility as (Quantitative Trait Locus) QTL markers for mapping genetic loci associated with environmental stress tolerance. As such, the  
15 sequences have utility in the identification of plants that exhibit an environmental stress tolerance phenotype from those that do not within a segregating population of plants. For example, to identify the region of the genome to which a particular glutaredoxin or thioredoxin nucleic acid sequence binds, genomic DNA could be digested with one or more restriction enzymes, and the fragments incubated with the glutaredoxin or thioredoxin nucleic acid, preferably with readily  
20 detectable labels. Binding of such a nucleic acid molecule to the genome fragment enables the localization of the fragment to the genome map and, when performed multiple times with different enzymes, facilitates a unique identifying pattern. Further, the nucleic acid molecules of the invention may be sufficiently homologous to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map.

25 The genetics of quantitative traits associated to DNA markers has been used extensively in plant breeding for more than a decade (Tansgley et al., 1989 Biotechnology 7:257-264). The principle consists of using segregating lines derived from two homozygous parents and mapping these progeny with markers to link each marker to at least another one (saturated map), after which a statistical relationship between the quantitative trait value and the genotype at each  
30 marker is determined. A significant link of a locus to the trait means that at least one gene that in the vicinity of the marker contributes part of the phenotype variability. By definition, this locus is called a quantitative trait locus (QTL). In such a case, the gene becomes a candidate gene for explaining part of the observed phenotype and methods to identify and clone these genes have

been described (Yano M, 2001. *Current Opinion in Plant Biology* 4:130-135). An observed correlation between a QTL and a gene location is likely to be causal, and therefore much more informative than a physiological correlation. This approach was applied to biochemical traits related to carbohydrate metabolism in maize leaves (Causse M., et al., 1995. *Molecular Breeding* 1:259-272).

This invention uses an alternative approach to the classical method. The approach of this invention is to use the QTL methodology linking a gene or locus known to be associated with the phenotype as a screening method. The marker may be associated with either the DNA sequences or the expression level of the gene, e.g. quantity of a specific mRNA molecule. In this instance, the marker serves as a convenient genetic means to identify individuals with the stress tolerance phenotype within a population of individuals that lack the phenotype. This method has utility when the phenotype is often difficult or expensive to detect or quantitative.

Many traits including tolerance of environmental stress and yield are associated with multiple genes and are therefore considered quantitative traits. This means that more than one marker or genetic locus is associated with the phenotype. In many instances, it is necessary to stack the various loci related to a phenotype. This is accomplished in standard plant breeding methods by cross-pollinating two parents with different loci (markers) contributing to the phenotype and selecting those progeny that have both markers. This process of breeding and selecting can be repeated multiple times to combine all loci into one progeny.

This invention provides markers of specific genetic loci that are associated with tolerance of abiotic environmental stress. The DNA sequences in SEQ IDs 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants may be used in the identification and selection of stress tolerant plants. These plants, their seeds and varieties derived from them would not contain transgenes but would contain alleles or genetic loci representing natural genetic diversity and thereby exhibit increased tolerance of abiotic environmental stress.

Growing the modified plant under less than suitable conditions and then analyzing the growth characteristics and/or metabolism can assess the effect of the genetic modification in plants on stress tolerance. Such analysis techniques are well known to one skilled in the art, and include dry weight, wet weight, protein synthesis, carbohydrate synthesis, lipid synthesis, evapotranspiration rates, general plant and/or crop yield, flowering, reproduction, seed setting, root growth, respiration rates, photosynthesis rates, etc. (Applications of HPLC in Biochemistry in: *Laboratory Techniques in Biochemistry and Molecular Biology*, vol. 17; Rehm et al., 1993

Biotechnology, vol. 3, Chapter III: Product recovery and purification, page 469-714, VCH: Weinheim; Belter, P.A. et al., 1988 Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S., 1992 Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D., 1988 Biochemical separations, in: Ulmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

The engineering of one or more Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin genes of the invention may also result in Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin proteins having altered activities which indirectly impact the stress response and/or stress tolerance of plants. For example, the normal biochemical processes of metabolism result in the production of a variety of products (e.g., hydrogen peroxide and other reactive oxygen species) which may actively interfere with these same metabolic processes (for example, peroxynitrite is known to react with tyrosine side chains, thereby inactivating some enzymes having tyrosine in the active site (Groves, J.T., 1999 Curr. Opin. Chem. Biol. 3(2):226-235). By optimizing the activity of one or more Oxidoreductase Stress-Related Protein, particularly glutaredoxin or thioredoxin enzymes of the invention, it may be possible to improve the stress tolerance of the cell.

Additionally, the sequences disclosed herein, or fragments thereof, can be used to generate knockout mutations in the genomes of various plant cells (Girke, T., 1998 The Plant Journal 15:39-48). The resultant knockout cells can then be evaluated for their ability or capacity to tolerate various stress conditions, their response to various stress conditions, and the effect on the phenotype and/or genotype of the mutation. For other methods of gene inactivation see U.S. Patent No. 6004804 "Non-Chimeric Mutational Vectors" and Puttaraju et al., 1999 Spliceosome-mediated RNA *trans*-splicing as a tool for gene therapy Nature Biotechnology 17:246-252.

Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it

is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

5

The invention also pertains the use of ORSRP encoding nucleic acid selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof for preparing a plant cell with increased environmental stress tolerance. The said sequences can also be used for preparing a plant with increased environmental stress tolerance. Object of the invention is further the use of ORSRP encoding nucleic acid selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof or parts thereof as DNA markers for selection of plants with increased tolerance to environmental stress. The said ORSRP encoding nucleic acid selected from the group comprising of SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof or parts thereof can also be used as Quantitative Trait Locus (QTL) markers for mapping genetic loci associated with environmental stress tolerance.

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**Example 1**

**Engineering stress-tolerant Arabidopsis plants by over-expressing glutaredoxin or thioredoxin genes.**

**5 Gene cloning and Transformation of Arabidopsis thaliana**

**Amplification** The standard protocol of *Pfu* DNA polymerase or a *Pfu/Taq* DNA polymerase mix was used for the amplification procedure. Amplified ORF fragments were analysed by gel electrophoresis. Each primer consists of a universal 5' end and ORF specific 3' end whereby the universal sequences differ for the forward and reverse primers (forward primer sequence contains a *EcoRI* and the reverse primer sequence a *SmaI* restriction site) allowing a unidirectional cloning success.

Amplification using the protocol of *Pfu* or *Herculase* DNA polymerase (Stratagene). Conditions: 1x PCR buffer [20 mM Tris-HCl (pH 8.8), 2 mM MgSO<sub>4</sub>, 10 mM KCl, 10mM (NH<sub>4</sub>)SO<sub>4</sub>, 0.1 % Triton X-100, 0.1 mg/ml BSA], 100 ng genomic DNA *Saccharomyces cerevisiae* (S288C), 50 pmol forward primer, 50 pmol reverse primer, 2.5 u *Pfu* or *Herculase* DNA polymerase. 1st cycle for 3' at 94 °C, followed by 25 cycles for 30'' at 94°C, 30'' 55 °C and 5-6' 72 °C, followed by 1 cycle for 6-10' at 72 °C, final for 4 °C at ∞.

20 YDR513w primer forward:

**GGAATTCCAGCTGACCACCATGGAGACCAATTTTCCTTCGACT**

YDR513w primer reverse:

**GATCCCCGGGAATTGCCATGCTATTGAAATACCGGCTTCAATATT**

25 YER174c primer forward:

**GGAATTCCAGCTGACCACCATGACTGTGGTTGAAATAAAAAGCC**

YER174c primer reverse:

**GATCCCCGGGAATTGCCATGTTACTGTAGAGCATGTTGGAAATATT**

**Vector preparation.** The preferred binary vector 1bxbigResgen, which is based on the modified pPZP binary vector backbone (comprising the kanamycin-gene for bacterial selection; Hajukiewicz, P. et al., 1994, plant Mol. Biol., 25: 989-994) carried the selection marker bar-gene (De Block et al., 1987, EMBO J. 6, 2513-2518) driven by the mas1'promotor (Velten et al., 1984, EMBO J. 3, 2723-2730; Mengiste, Amedeo and Paszkowski, 1997, Plant J., 12, 945-948) on its T-DNA. In addition the T-DNA contained the strong double 35S promotor (Kay et al., 1987, Science 236, 1299-1302) in front of a cloning cassette followed by the nos-terminator (Depicker A. Stachel S. Dhaese P. Zambryski P. Goodman HM. Nopaline synthase: transcript mapping and DNA sequence. Journal of Molecular & Applied Genetics. 1(6):561-73, 1982.). The cloning cassette consists of the following sequence:

5'-GGAATTCCAGCTGACCACCATGGCAATTCCCGGGGATC-3'

Other selection marker systems, like the AHAS marker or other promotors, e.g. superpromotor (Ni-Min et al., Plant Journal, 1995, 7(4): 661-676), Ubiquitin promotor (Callis et al., J. Biol. Chem., 1990, 265: 12486-12493; US 5,510,474; US 6,020,190; Kawalleck et al., Plant. Molecular Biology, 1993, 21: 673-684) or 34S promotor (GenBank Accession numbers M59930 and X16673) were similar useful for the instant invention and are known to a person skilled in the art. The vector was linearised with *EcoRI* and *SmaI* using the standard protocol provided by the supplier (MBI Fermentas, Germany) and purified using Qiagen columns (Qiagen, Hilden, Germany).

**Ligation and transformation** Present ORF fragments (~ 100 ng) were digested by *EcoRI* and *SmaI* using the standard protocol provided by the supplier (MBI Fermentas, Germany), purified using Qiagen columns (Qiagen, Hilden, Germany) and were ligated into the cloning cassette of the binary vector systems (~ 30 ng) using standard procedures (Maniatis et al.).

Ligation products were transformed into *E.coli* (DH5alpha) using a standard heat shock protocol (Maniatis et al.). Transformed colonies were grown on LB media and selected by respective antibiotica (Km) for 16h at 37 °C ÜN.

**Plasmidpreparation** Plasmids were prepared using standard protocol (Qiagen Hilden, Germany).

**Transformation of Agrobacteria** Plasmids were transformed into *Agrobacterium tumefaciens* (GV3101pMP90; Koncz and Schell, 1986 Mol. Gen. Genet. 204:383-396) using heat shock or

electroporation protocols. Transformed colonies were grown on YEP media and selected by respective antibiotika (Rif/Gent/Km) for 2d at 28 °C ÜN. These agrobacteria cultures were used for the plant transformation.

5

*Arabidopsis thaliana* was grown and transformed according to standard conditions (Bechtold 1993 (Bechtold, N., Ellis, J., Pelletier, G. 1993. *In planta Agrobacterium* mediated gene transfer by infiltration of *Arabidopsis thaliana* plants C.R. Acad.Sci.Paris. 316:1194-1199); Bent et al. 1994 (Bent, A., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B.J. 1994; PPCS2 of *Arabidopsis thaliana*: A leucin-rich repeat class of plant disease resistant genes; Science 265:1856-1860).

Transgenic *A. thaliana* plants were grown individually in pots containing a 4:1 (v/v) mixture of soil and quartz sand in a York growth chamber. Standard growth conditions were: photoperiod of 16 h light and 8 h dark, 20°C, 60% relative humidity, and a photon flux density of 150 µE. To induce germination, sown seeds were kept at 4°C, in the dark, for 3 days. Plants were watered daily until they were approximately 3 weeks old at which time drought was imposed by withholding water. Coincidentally, the relative humidity was reduced in 10% increments every second day to 20%. After approximately 12 days of withholding water, most plants showed visual symptoms of injury, such as wilting and leaf browning, whereas tolerant plants were identified as being visually turgid and healthy green in color. Plants were scored for symptoms of drought injury in comparison to neighbouring plants for 3 days in succession.

Three successive experiments were conducted. In the first experiment, 10 independent T2 lines were sown for each gene being tested. The percentage of plants not showing visual symptoms of injury was determined. In the second experiment, the lines that had been scored as tolerant in the first experiment were put through a confirmation screen according to the same experimental procedures. In this experiment, 10 plants of each tolerant line were grown and treated as before. In the third experiment, at least 5 replicates of the most tolerant line were grown and treated as before. The average and maximum number of days of drought survival after wild-type control had visually died and the percentage tolerant plants was determined. Additionally measurements of chlorophyll fluorescence were made in stressed and non-stressed plants using a Mini-PAM (Heinz Walz GmbH, Effeltrich, Germany).

In the first experiment, after 12 days of drought, the control, non-transgenic *Arabidopsis thaliana* and most transgenic lines expressing other transgenes in the test showed extreme visual symptoms of stress including necrosis and cell death. Several plants expressing the YER174C

(=ORF737; SEQ ID No. 7) gene and the YDR513W (=ORF809; SEQ ID No. 3) gene retained viability as shown by their turgid appearance and maintenance of green color. Several independent transgenic lines, in the case of both the YER174C and the YDR513W genes, did not become necrotic for at least 3 days after the control plants had died (Table 2 and 3).

- 5        The second experiment compared a smaller number of independent transgenic lines for each gene but a greater number of progeny within each independent transformation event. This experiment confirmed the previous results. Those lines containing the YER174C gene (Table 2) did not become necrotic for 1-2 days after the controls and in the case of the YDR513W gene, 2-3 days after the controls (Table 3).

10

Table 2: Drought tolerance of transgenic *Arabidopsis thaliana* expressing the YER174C gene after imposition of drought stress on 3 week old plants. Control plants showed extensive visual symptoms of injury on day 12 and were considered dead.

Experiment	Plant	Percent survival		
		Day 13	Day 14	Day 15
1	Control	0	0	0
	Transgenic 737	60	40	20
2	Control	0	0	0
	Transgenic 737-1	22	22	0
	Transgenic 737-3	50	0	0

15

Table 3: Drought tolerance of transgenic *Arabidopsis thaliana* expressing the YDR513W gene after imposition of drought stress on 3 week old plants. Control plants showed extensive visual symptoms of injury on day 12 and were considered dead.

Experiment	Plant	Percent survival		
		Day 13	Day 14	Day 15
1	Control	0	0	0
	Transgenic 809	50	33	33
2	Control	0	0	0

Transgenic 809-5	25	13	13
Transgenic 809-8	50	25	0

In the third experiment, one transgenic line from each gene was tested using a even larger number of plants. In line 737-3 expressing the YER174C gene, necrosis did not occur on average until 1.1 days after the controls and 2 of the 22 plants tested did not show necrosis until 4 days later (Table 4). Similarly, line 809-8 expressing the YDR513W gene survived on average 3.1 days longer than the control and 1 plant survived for 6 days longer later (Table 4). Other independent transgenic lines for both genes showed greater survival than the non-transgenic plants in this experiment.

Chlorophyll fluorescence measurements of photosynthetic yield confirmed that 12 days of drought stress completely inhibited photosynthesis in the control plants, but the transgenic line 809-8 maintained its photosynthetic function longer (Table 5).

Table 4: Relative drought tolerance of *Arabidopsis thaliana* transgenic line 737-3 expressing the YER174C gene and line 809-8 expressing the YDR513W gene after imposition of drought stress on 3 week old plants in comparison to non-transgenic control plants. Control plants showed extensive visual symptoms of injury on day 12 and were considered dead.

	737-3	809-8
Number of plants tested	22	7
Duration of survival after control (days)	1.1	3.1
Maximal duration of survival (number of plants)	3 (2)	6 (1)

Table 5: Effect of drought stress on photosynthetic yield as determined by chlorophyll fluorescence ( $\pm$  std deviation) of *Arabidopsis thaliana* control and transgenic line 809-8 expressing the YDR513W gene.

Days of drought	Control	Transgenic line 809-8
0	765 $\pm$ 29	723 $\pm$ 29
5	794 $\pm$ 36	781 $\pm$ 25
10	412 $\pm$ 194	660 $\pm$ 121
12	54 $\pm$ 83	411 $\pm$ 305

## Example 2

### Isolation and Characterization of plant glutaredoxin genes

5           ORF 737 and 809 correspond to yeast, *Saccharomyces cerevisiae*, genes for glutaredoxin4 (GRX4) and glutaredoxin2 (GRX2), respectively, that contain a pair of cysteine amino acids at the putative active site of the protein (Grant CM. 2000. Molecular Microbiology 39: 533-541; Grant CM et al., 2001. Biochimica et Biophysica Acta - Gene Structure & Expression 1490: 33-42). Grx3, Grx4, and Grx5 is a subfamily of yeast glutaredoxins that contain  
10 a single cysteine residue at the putative active site (Rodriguez-Manzanique et al., 1999. Molecular & Cellular Biology 19: 8180-8190). *Saccharomyces cerevisiae* also contains two gene pairs for thioredoxins (TRX1, TRX2) (Draculic et al., 2000. Molecular Microbiology 36: 1167-1174). These gene sequences are listed in GenBank under the accession numbers listed in Table 6.

15           The sequence of GRX2 and GRX4 was used to identify related gene sequences in *Arabidopsis thaliana* by Blast analysis (Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990 J Mol Biol 215(3):403-10). The results identified related sequences with  $E < 10^{-10}$  as shown in Table 6, where E is defined as the expectancy value, or the statistical probability that the sequence appears in the database at random.. A similar analysis was done on a three libraries of  
20 expressed sequence tags (ESTs) from *Brassica napus* cv. "AC Excel", "Quantum" and "Cresor" (canola) and *Oryza sativa* cv. Nippon-Barre (a japonica rice). The search identified several Brassica and rice glutaredoxin cDNA sequences with  $E < 10^{-10}$  (Table 6).

          The yeast and plant cDNA sequences were translated into a predicted amino acid sequences and the relationship among the amino acid sequences was determined by sequence  
25 alignment and block alignment using the ClustalW algorithm in Vector NTI ver7. The glutaredoxin and thioredoxin genes were separated into four subfamilies based on this alignment as shown in Figure 1. The glutaredoxin family is characterized by the standard glutaredoxin domain defined in the Prosite database as an amino acid motif with the consensus sequence [LIVMD]-[FYSA]-x(4)-C-[PV]-[FYWH]-C-x(2)-[TAV]-x(2,3)-[LIV]. Most sequences show the  
30 characteristic two cysteines that when reduced form either two thiol groups or when oxidized form a disulfide bond. Other proteins in this family have only a single C at this site.

          Subfamily 1 contains the yeast genes GRX1 and GRX2 (figures 2-4). Domain 1 has the core sequence [VI]-[VF]-[VI]-X-[SA]-K-[TS]-[WY]-C-[PGS]-[YF]-[CS]. OZ1116C26232 and

AtQ95K75 lack the C-X-X-C disulfide site and instead have a single C at this site. Domain 2 contains a motif defined as G-Q-X-T-V-P-N-[VI]-[FY]-[VI]-X-G-[KN]-H-I-G-G-[CN].

Subfamily 2 contains both glutaredoxin GRX3 and GRX4 and thioredoxin THX1 and THX2 sequences (figures 5-7). This family has a region of homology comprising two domains. In most sequences the domains are continuous, except in GRX3 and GRX4 in which the two domains are separated by two amino acids. Domain 1 has a core sequence of [VI]-V-[VL]-X-F-X-[TA]-X-W-[CA]-X-[PA]-[CS]-K. The region [CA]-X(2)-[CS] contains C at position 1 or 4 or both. Domain 2 is a region of similarity that has a core sequence of F-X(2)-[VI]-[ED]-[AV]-[ED]-E-X(2)-[ED]-[IV].

Subfamily 3 contains GRX5 and three plant sequences that have a single C amino acid at the putative active site (figures 8-11). The core sequence of domain 1 is V-[VM]-X(3)-K-G-X(4)-P-X-C-G-F-S. Domain 2 is defined by the sequence Q-[LI]-[FY]-[VI]-X-[GK]-E-[FL]-X-G-G-[CS]-D-[IV].

Subfamily 4 does not have any members from yeast and is comprised of 5 plant sequences that have two domains of homology (figures 11-13). Domain 1 has a core sequence similar to subfamily 1 that is [VI]-V-I-F-S-K-S-Y-C-P-Y-C. Domain 2 has two regions with common sequences of V-V-E-L-D-X-R-E-D-G and V-G-R-R-T-V-P-Q-V-F-[VI]-[NH]-G-K-H-[LI]-G-G-S-D-D.

A representative of each subfamily was selected and the full length coding sequence was ligated into a plant transformation vector using standard molecular biology techniques as described in Example 1. The coding sequence was inserted at the 3' end of a constitutive promoter to control expression in plants. The vector was transferred to *Agrobacterium tumefaciens* and this strain was used to transform *Arabidopsis thaliana* as described in Example 1. Transgenic plants were grown and treated with drought stress as described in Example 1. Those plants that contained the glutaredoxin/thioredoxin transgene from subfamilies 1, 2 and 3 were more tolerant of the drought treatment than the control, non-transgenic plants.

Table 6. Summary of yeast and plant glutaredoxin coding sequences.  
Query specifies the ORF sequence used for the Blast search

source	SubFamily	query	Gene ID	GenBank Accession	Nucleotide SEQ ID No.	Amino acid SEQ ID No.
Yeast	1		GRX1	X59720	1	2
	1	809	GRX2	U18922	3	4
	2		GRX3	Z47746	5	6
	2	737	GRX4	U33057	7	8
	3		GRX5	U39205	9	10
	2		THX1	M59168	11	12
	2		THX2	M59169	13	14
Brassica	1	809	BN1106C12219	NA	15	16
	4	809	BN1106C21909	NA	17	18
	1	809	BN1106C2202	NA	19	20
	4	809	BN1106C2582	NA	21	22
	2	737	BN1106C23043	NA	23	24
Arabidopsis	1	809	AtQ9FM49	AB009051	25	26
	1	809	AtQ9FNE2	AB006702	27	28
	4	809	AtQ9FVX1	NM 106386	29	30
	4	809	AtQ9M457	ATH271472	31	32
	1	809	AtQ9SK75	AY094445	33	34
	3	737	AtQ9LW13	AY087154	35	36
	3	737	AtQ9SV38	AY078020	37	38
	3	737	AtO80451	AY086273	39	40
	2	737	AtO65541	NM 119410	41	42
	2	737	AtQ9ZPH2	AY058202	43	44
Rice	4	809	OZ1116C12744	NA	45	46
	1	809	OZ1116C2194	X77150	47	48
	1	809	OZ1116C26232	NA	49	50

5

NA- not available; sequence is not in a GenBank database

### Example 3

10 Engineering stress-tolerant Arabidopsis plants by over-expressing glutaredoxin or thioredoxin genes using stress-inducible and tissue-specific promoters.

Transgenic Arabidopsis plants were created as in example 1 to express the glutaredoxin and thioredoxin transgenes under the control of either a tissue-specific or stress-inducible promoter. Constitutive expression of a transgene may cause deleterious side effects. Stress  
15 inducible expression was achieved using promoters selected from those listed above in Table 1.



T2 generation plants were produced and treated with drought stress in two experiments. For the first drought experiment, the plants were deprived of water until the plant and soil were desiccated. At various times after withholding water, a normal watering schedule was resumed and the plants were grown to maturity. Seed yield was determined as g seeds per plant. At an equivalent degree of drought stress, tolerant plants were able to resume normal growth and produced more seeds than non-transgenic control plants. Proline content of the leaves and stomatal aperture were also measured at various times during the drought stress. Tolerant plants maintained a lower proline content and a greater stomatal aperture than the non-transgenic control plants.

An alternative method to impose water stress on the transgenic plants was by treatment with water containing an osmolyte such as polyethylene glycol (PEG) at specific water potential. Since PEG may be toxic, the plants were given only a short term exposure and then normal watering was resumed. As above, seed yields were measured from the mature plants. The response was measured during the stress period by physical measurements, such as stomatal aperture or osmotic potential, or biochemical measurements, such as accumulation of proline. Tolerant plants had higher seed yields, maintained their stomatal aperture and showed only slight changes in osmotic potential and proline levels, whereas the susceptible non-transgenic control plants closed their stomata and exhibited increased osmotic potential and proline levels.

The transgenic plants with a constitutive promoter controlling transcription of the transgene were compared to those plants with a drought-inducible promoter in the absence of stress. The results indicated that the metabolite and gene expression changes noted in examples 2 and 3 did not occur when plants with the stress-inducible promoter were grown in the absence of stress. These plants also had higher seed yields than those with the constitutive promoter.

#### **Example 4**

#### **Inheritance and segregation of drought tolerance with the glutaredoxin and thioredoxin transgenes.**

Transgenic Arabidopsis plants in the T2 generation were analyzed by PCR to confirm the presence of T-DNA. These results were confirmed by Southern hybridization in which DNA is electrophoresed on a 1% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a

digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer. Homozygous lines with single insertions of T-DNA were selected for cross-pollination experiments.

5 A homozygous line with the glutaredoxin transgene (GG) was cross-pollinated with a homozygous line with the thioredoxin transgene (TT). Since the transgenes are not at the same locus, the F1 progeny were heterozygous (G-T-). The F2 progeny segregated in a 9: 3: 3: 1 ratio of double transformants containing both transgenes, to single transformants containing either G or T, and nulls containing neither transgene. The genotype of the progeny was determined by PCR analysis for each of the transgenes. Homozygous lines of each genotype GGTT, GG--, --TT, and -  
10 --- were identified by quantitative PCR and confirmed by inheritance patterns of the transgenes.

Homozygous lines were subjected to drought stress, metabolite analysis and expression profiling as described in examples 1, 2, 3 and 4. The transgenic lines were more drought tolerant than the null line, had altered metabolite levels consistent with the observations in example 2 and altered gene expression patterns consistent with the observations in example 3.

15

#### Example 5

**Over-expression of glutaredoxin or thioredoxin genes provides tolerance of multiple abiotic stresses.**

Plants that exhibit tolerance of one abiotic stress often exhibit tolerance of another  
20 environmental stress or an oxygen free radical generating herbicide. This phenomenon of cross-tolerance is not understood at a mechanistic level (McKersie and Leshem, 1994). Nonetheless, it is reasonable to expect that plants exhibiting enhanced drought tolerance due to the expression of a transgene might also exhibit tolerance of low temperatures, freezing, salt, air pollutants such as ozone, and other abiotic stresses. In support of this hypothesis, the expression of several genes are  
25 up or down-regulated by multiple abiotic stress factors including cold, salt, osmoticum, ABA, etc (e.g. Hong et al. (1992) Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. *Plant Mol Biol* 18: 663-674; Jagendorf and Takabe (2001) Inducers of glycinebetaine synthesis in barley. *Plant Physiol* 127: 1827-1835); Mizoguchi et al. (1996) A gene encoding a mitogen-activated protein kinase kinase is induced simultaneously with genes for  
30 a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 93: 765-769; Zhu (2001) Cell signaling under salt, water and cold stresses. *Curr Opin Plant Biol* 4: 401-406).

To determine salt tolerance, seeds of *Arabidopsis thaliana* were sterilized (100% bleach, 0.1% TritonX for five minutes two times and rinsed five times with ddH<sub>2</sub>O). Seeds were plated on non-selection media (1/2 MS, 0.6% phytagar, 0.5g/L MES, 1% sucrose, 2 µg/ml benamyl). Seeds are allowed to germinate for approximately ten days. At the 4-5 leaf stage, transgenic plants were potted into 5.5cm diameter pots and allowed to grow (22°C, continuous light) for approximately seven days, watering as needed. To begin the assay, two liters of 100 mM NaCl and 1/8 MS was added to the tray under the pots. To the tray containing the control plants, three liters of 1/8 MS was added. The concentrations of NaCl supplementation were increased stepwise by 50 mM every 4 days up to 200 mM. After the salt treatment with 200 mM, fresh and dry weights of the plants as well as seed yields were determined.

To determine cold tolerance, seeds of the transgenic and cold lines were germinated and grown for approximately 10 days to the 4-5 leaf stage as above. The plants were then transferred to cold temperatures (5°C) and grown through the flowering and seed set stages of development. Photosynthesis was measured using chlorophyll fluorescence as an indicator of photosynthetic fitness and integrity of the photosystems. Seed yield and plant dry weight were measured as an indicator of plant biomass production.

Plants that had tolerance to salinity or cold had higher seed yields, photosynthesis and dry matter production than susceptible plants.

## Example 6

### Engineering stress-tolerant alfalfa plants by over-expressing glutaredoxin or thioredoxin genes.

A regenerating clone of alfalfa (*Medicago sativa*) was transformed using the method of (McKersie et al., 1999 Plant Physiol 119: 839–847). Regeneration and transformation of alfalfa is genotype dependent and therefore a regenerating plant is required. Methods to obtain regenerating plants have been described. For example, these can be selected from the cultivar Rangelander (Agriculture Canada) or any other commercial alfalfa variety as described by Brown DCW and A Atanassov (1985. Plant Cell Tissue Organ Culture 4: 111-112). Alternatively, the RA3 variety (University of Wisconsin) has been selected for use in tissue culture (Walker et al., 1978 Am J Bot 65:654–659).

Petiole explants were cocultivated with an overnight culture of *Agrobacterium tumefaciens* C58C1 pMP90 (McKersie et al., 1999 Plant Physiol 119: 839–847) or LBA4404

containing a binary vector. Many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium Protocols. Methods in Molecular Biology* vol 44, pp 47-62, Gartland KMA and MR Davey eds. Humana Press, Totowa, New Jersey). Many are based on the vector pBIN19 described by Bevan (*Nucleic Acid Research*. 1984. 12:8711-8721) that includes a plant gene expression cassette flanked by the left and right border sequences from the Ti plasmid of *Agrobacterium tumefaciens*. A plant gene expression cassette consists of at least two genes – a selection marker gene and a plant promoter regulating the transcription of the cDNA or genomic DNA of the trait gene. Various selection marker genes can be used including the Arabidopsis gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patents 57673666 and 6225105). Similarly, various promoters can be used to regulate the trait gene that provides constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

The explants were cocultivated for 3 d in the dark on SH induction medium containing 288 mg/ L Pro, 53 mg/ L thioproline, 4.35 g/ L K<sub>2</sub>SO<sub>4</sub>, and 100 µm acetosyringinone. The explants were washed in half-strength Murashige-Skoog medium (Murashige and Skoog, 1962) and plated on the same SH induction medium without acetosyringinone but with a suitable selection agent and suitable antibiotic to inhibit *Agrobacterium* growth. After several weeks, somatic embryos were transferred to BOi2Y development medium containing no growth regulators, no antibiotics, and 50 g/ L sucrose. Somatic embryos were subsequently germinated on half-strength Murashige-Skoog medium. Rooted seedlings were transplanted into pots and grown in a greenhouse.

The T0 transgenic plants were propagated by node cuttings and rooted in Turface growth medium. The plants were defoliated and grown to a height of about 10 cm (approximately 2 weeks after defoliation). The plants were then subjected to drought stress in two experiments.

For the first drought experiment, the seedlings received no water for a period up to 3 weeks at which time the plant and soil were desiccated. At various times after withholding water, a normal watering schedule was resumed. At one week after resuming watering, the fresh and dry weights of the shoots was determined. At an equivalent degree of drought stress, tolerant plants were able to resume normal growth whereas susceptible plants had died or suffered significant injury resulting in less dry matter. Proline content of the leaves and stomatal aperture were also measured at various times during the drought stress. Tolerant plants maintained a lower proline content and a greater stomatal aperture than the non-transgenic control plants.

An alternative method to impose water stress on the transgenic plants was by treatment with a solution at specific water potential, containing an osmolyte such as polyethylene glycol (PEG). The PEG treatment was given to either detached leaves (e.g. Djilianov et al., 1997 Plant Science 129: 147-156) or to the roots (Wakabayashi et al., 1997 Plant Physiol 113: 967-973).

5 Since PEG may be toxic, the plants were given only a short term exposure. The response was measured as physical measurements such as stomatal aperture or osmotic potential, or biochemical measurements such as accumulation of proline. Tolerant plants maintained their stomatal aperture and showed only slight changes in osmotic potential, whereas the susceptible non-transgenic control plants closed their stomata and exhibited increased osmotic potential. In  
10 addition the changes in proline and other metabolites were less in the tolerant transgenic plants than in the non-transgenic control plants.

Tolerance of salinity and cold were measured using methods as described in example 5. Plants that had tolerance to salinity or cold had higher seed yields, photosynthesis and dry matter production than susceptible plants.

#### 15 **Example 7**

##### **Engineering stress-tolerant ryegrass plants by over-expressing glutaredoxin or thioredoxin genes.**

Seeds of several different ryegrass varieties may be used as explant sources for transformation, including the commercial variety Gunne available from Svalof Weibull seed  
20 company or the variety Affinity. Seeds were surface-sterilized sequentially with 1% Tween-20 for 1 minute, 100 % bleach for 60 minutes, 3 rinses with 5 minutes each with de-ionized and distilled H<sub>2</sub>O, and then germinated for 3-4 days on moist, sterile filter paper in the dark. Seedlings were further sterilized for 1 minute with 1% Tween-20, 5 minutes with 75% bleach, and rinsed 3 times with ddH<sub>2</sub>O, 5 min each.

25 Surface-sterilized seeds were placed on the callus induction medium containing Murashige and Skoog basal salts and vitamins, 20 g/l sucrose, 150 mg/l asparagine, 500 mg/l casein hydrolysate, 3 g/l Phytigel, 10 mg/l BAP, and 5 mg/l dicamba. Plates were incubated in the dark at 25C for 4 weeks for seed germination and embryogenic callus induction

30 After 4 weeks on the callus induction medium, the shoots and roots of the seedlings were trimmed away, the callus was transferred to fresh media, maintained in culture for another 4 weeks, and then transferred to MSO medium in light for 2 weeks. Several pieces of callus (11-17 weeks old) were either strained through a 10 mesh sieve and put onto callus induction medium, or

cultured in 100 ml of liquid ryegrass callus induction media (same medium as for callus induction with agar) in a 250 ml flask. The flask was wrapped in foil and shaken at 175 rpm in the dark at 23 C for 1 week. Sieving the liquid culture with a 40-mesh sieve collected the cells. The fraction collected on the sieve was plated and cultured on solid ryegrass callus induction medium for 1  
5 week in the dark at 25C. The callus was then transferred to and cultured on MS medium containing 1% sucrose for 2 weeks.

Transformation can be accomplished with either *Agrobacterium* or with particle bombardment methods. An expression vector is created containing a constitutive plant promoter and the cDNA of the gene in a pUC vector. The plasmid DNA was prepared from *E. coli* cells  
10 using with Qiagen kit according to manufacturer's instruction. Approximately 2 g of embryogenic callus was spread in the center of a sterile filter paper in a Petri dish. An aliquot of liquid MSO with 10 g/l sucrose was added to the filter paper. Gold particles (1.0 µm in size) were coated with plasmid DNA according to method of Sanford et al., 1993 and delivered to the embryogenic callus with the following parameters: 500 µg particles and 2 µg DNA per shot, 1300 psi and a  
15 target distance of 8.5 cm from stopping plate to plate of callus and 1 shot per plate of callus.

After the bombardment, calli were transferred back to the fresh callus development medium and maintained in the dark at room temperature for a 1-week period. The callus was then transferred to growth conditions in the light at 25C to initiate embryo differentiation with the appropriate selection agent, e.g. 250 nM Arsenal, 5 mg/l PPT or 50 mg/L kanamycin. Shoots  
20 resistant to the selection agent appeared and once rooted were transferred to soil.

Samples of the primary transgenic plants (T0) are analyzed by PCR to confirm the presence of T-DNA. These results are confirmed by Southern hybridization in which DNA is electrophoresed on a 1% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a  
25 digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer.

Transgenic T0 ryegrass plants were propagated vegetatively by excising tillers. The transplanted tillers were maintained in the greenhouse for 2 months until well established. The shoots were defoliated and allowed to grow for 2 weeks.

The first drought experiment was conducted in a manner similar to that described in  
30 example 5. The seedlings received no water for a period up to 3 weeks at which time the plant and soil were desiccated. At various times after withholding water, a normal watering schedule was resumed. At one week after resuming watering, the lengths of leaf blades, and the fresh and dry weights of the shoots was determined. At an equivalent degree of drought stress, tolerant plants were able to resume normal growth whereas susceptible plants had died or suffered

significant injury resulting in shorter leaves and less dry matter. Proline content of the leaves and stomatal aperture were also measured at various times during the drought stress. Tolerant plants maintained a lower proline content and a greater stomatal aperture than the non-transgenic control plants.

- 5        A second experiment imposing drought stress on the transgenic plants was by treatment with a solution of PEG as described in the previous examples. Tolerance of salinity and cold were measured using methods as described in example 5. Plants that had tolerance to salinity or cold had higher seed yields, photosynthesis and dry matter production than susceptible plants.

## 10    **Example 8**

**Engineering stress-tolerant soybean plants by over-expressing glutaredoxin or thioredoxin genes.**

- 15        Soybean was transformed according to the following modification of the method described in the Texas A&M patent US 5,164,310. Several commercial soybean varieties are amenable to transformation by this method. The cultivar Jack (available from the Illinois Seed Foundation) is a commonly used for transformation. Seeds were sterilized by immersion in 70% (v/v) ethanol for 6 min and in 25 % commercial bleach (NaOCl) supplemented with 0.1% (v/v) Tween for 20 min, followed by rinsing 4 times with sterile double distilled water. Seven-day seedlings were propagated by removing the radicle, hypocotyl and one cotyledon from each seedling. Then, the epicotyl with one cotyledon was transferred to fresh germination media in petri dishes and incubated at 25°C under a 16-hr photoperiod (approx.  $100 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for three weeks. Axillary nodes (approx. 4 mm in length) were cut from 3 – 4 week-old plants. Axillary nodes were excised and incubated in *Agrobacterium* LBA4404 culture.

- 25        Many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium* Protocols. Methods in Molecular Biology vol 44, pp 47-62, Gartland KMA and MR Davey eds. Humana Press, Totowa, New Jersey). Many are based on the vector pBIN19 described by Bevan (Nucleic Acid Research. 1984. 12:8711-8721) that includes a plant gene expression cassette flanked by the left and right border sequences from the Ti plasmid of *Agrobacterium tumefaciens*. A plant gene expression cassette consists of at least two genes – a selection marker gene and a plant promoter regulating the transcription of the cDNA or genomic DNA of the trait gene. Various selection marker genes can be used including the Arabidopsis gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patents 57673666 and
- 30

6225105). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

5 After the co-cultivation treatment, the explants were washed and transferred to selection media supplemented with 500 mg/L timentin. Shoots were excised and placed on a shoot elongation medium. Shoots longer than 1 cm were placed on rooting medium for two to four weeks prior to transplanting to soil.

10 The primary transgenic plants (T0) were analyzed by PCR to confirm the presence of T-DNA. These results were confirmed by Southern hybridization in which DNA is electrophoresed on a 1% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer.

15 Tolerant plants had higher seed yields, maintained their stomatal aperture and showed only slight changes in osmotic potential and proline levels, whereas the susceptible non-transgenic control plants closed their stomata and exhibited increased osmotic potential and proline levels.

20 Tolerance of salinity and cold were measured using methods as described in example 5. Plants that had tolerance to salinity or cold had higher seed yields, photosynthesis and dry matter production than susceptible plants.

### Example 9

#### Engineering stress-tolerant Rapeseed/Canola plants by over-expressing glutaredoxin or thioredoxin genes.

25 Cotyledonary petioles and hypocotyls of 5-6 day-old young seedlings were used as explants for tissue culture and transformed according to Babic et al.(1998, Plant Cell Rep 17: 183-188). The commercial cultivar Westar (Agriculture Canada) is the standard variety used for transformation, but other varieties can be used.

30 *Agrobacterium tumefaciens* LBA4404 containing a binary vector was used for canola transformation. Many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium Protocols. Methods in Molecular Biology* vol 44, pp 47-62, Gartland KMA and MR Davey eds. Humana Press, Totowa, New Jersey). Many are based on the



vector pBIN19 described by Bevan (Nucleic Acid Research. 1984. 12:8711-8721) that includes a plant gene expression cassette flanked by the left and right border sequences from the Ti plasmid of *Agrobacterium tumefaciens*. A plant gene expression cassette consists of at least two genes – a selection marker gene and a plant promoter regulating the transcription of the cDNA or genomic DNA of the trait gene. Various selection marker genes can be used including the Arabidopsis gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patents 57673666 and 6225105). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

Canola seeds were surface-sterilized in 70% ethanol for 2 min., and then in 30% Clorox with a drop of Tween-20 for 10 min, followed by three rinses with sterilized distilled water. Seeds were then germinated in vitro 5 days on half strength MS medium without hormones, 1% sucrose, 0.7% Phytagar at 23°C, 16 hr. light. The cotyledon petiole explants with the cotyledon attached were excised from the *in vitro* seedlings, and inoculated with *Agrobacterium* by dipping the cut end of the petiole explant into the bacterial suspension. The explants were then cultured for 2 days on MSBAP-3 medium containing 3 mg/l BAP, 3% sucrose, 0.7% Phytagar at 23C, 16 hr light. After two days of co-cultivation with *Agrobacterium*, the petiole explants were transferred to MSBAP-3 medium containing 3 mg/l BAP, cefotaxime, carbenicillin, or timentin (300 mg/l) for 7 days, and then cultured on MSBAP-3 medium with cefotaxime, carbenicillin, or timentin and selection agent until shoot regeneration. When the shoots were 5 – 10 mm in length, they were cut and transferred to shoot elongation medium (MSBAP-0.5, containing 0.5 mg/l BAP). Shoots of about 2 cm in length were transferred to the rooting medium (MS0) for root induction.

Samples of the primary transgenic plants (T0) were analyzed by PCR to confirm the presence of T-DNA. These results were confirmed by Southern hybridization in which DNA is electrophoresed on a 1% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a digoxigenin-labelled probe by PCR, and used as recommended by the manufacturer.

The transgenic plants were then evaluated for their improved stress tolerance according to the method described in Example 5. Tolerant plants had higher seed yields, maintained their stomatal aperture and showed only slight changes in osmotic potential and proline levels, whereas the susceptible non-transgenic control plants closed their stomata and exhibited increased osmotic potential and proline levels.

Tolerance of salinity and cold were measured using methods as described in the previous example 5. Plants that had tolerance to salinity or cold had higher seed yields, photosynthesis and dry matter production than susceptible plants.

## 5 Example 10

### Engineering stress-tolerant corn plants by over-expressing glutaredoxin or thioredoxin genes.

Transformation of maize (*Zea Mays L.*) is performed with a modification of the method described by Ishida et al. (1996. Nature Biotech 14745-50). Transformation is genotype-dependent in corn and only specific genotypes are amenable to transformation and regeneration. The inbred line A188 (University of Minnesota) or hybrids with A188 as a parent are good sources of donor material for transformation (Fromm et al. 1990 Biotech 8:833-839), but other genotypes can be used successfully as well. Ears are harvested from corn plants at approximately 11 days after pollination (DAP) when the length of immature embryos is about 1 to 1.2 mm. Immature embryos are co-cultivated with *Agrobacterium tumefaciens* that carry "super binary" vectors and transgenic plants are recovered through organogenesis. The super binary vector system of Japan Tobacco is described in WO patents WO94/00977 and WO95/06722. Vectors were constructed as described. Various selection marker genes can be used including the maize gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patent 6025541). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

Excised embryos are grown on callus induction medium, then maize regeneration medium, containing imidazolinone as a selection agent. The Petri plates are incubated in the light at 25°C for 2-3 weeks, or until shoots develop. The green shoots are transferred from each embryo to maize rooting medium and incubated at 25°C for 2-3 weeks, until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the imidazolinone herbicides and which are PCR positive for the transgenes.

The T1 transgenic plants were then evaluated for their improved stress tolerance according to the method described in Example 5. The T1 generation of single locus insertions of

the the T-DNA will segregate for the transgene in a 3:1 ratio. Those progeny containing one or two copies of the transgene are tolerant of the imidazolinone herbicide, and exhibit greater tolerance of drought stress than those progeny lacking the transgenes. Tolerant plants had higher seed yields, maintained their stomatal aperture and showed only slight changes in osmotic potential and proline levels, whereas the susceptible non-transgenic control plants closed their stomata and exhibited increased osmotic potential and proline levels. Homozygous T2 plants exhibited similar phenotypes.

Tolerance of salinity and cold were measured using methods as described in the previous example 5. Plants that had tolerance to salinity or cold had higher seed yields, photosynthesis and dry matter production than susceptible plants.

### Example 11

#### Engineering stress-tolerant wheat plants by over-expressing glutaredoxin or thioredoxin genes.

Transformation of wheat is performed with the method described by Ishida et al. (1996 Nature Biotech. 14745-50). The cultivar Bobwhite (available from CYMMIT, Mexico) is commonly used in transformation. Immature embryos are co-cultivated with *Agrobacterium tumefaciens* that carry "super binary" vectors, and transgenic plants are recovered through organogenesis. The super binary vector system of Japan Tobacco is described in WO patents WO94/00977 and WO95/06722. Vectors were constructed as described. Various selection marker genes can be used including the maize gene encoding a mutated acetohydroxy acid synthase (AHAS) enzyme (US patent 6025541). Similarly, various promoters can be used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription. In this example, the 34S promoter (GenBank Accession numbers M59930 and X16673) was used to provide constitutive expression of the trait gene.

After incubation with *Agrobacterium*, the embryos are grown on callus induction medium, then regeneration medium, containing imidazolinone as a selection agent. The Petri plates are incubated in the light at 25°C for 2-3 weeks, or until shoots develop. The green shoots are transferred from each embryo to rooting medium and incubated at 25°C for 2-3 weeks, until roots develop. The rooted shoots are transplanted to soil in the greenhouse. T1 seeds are produced from plants that exhibit tolerance to the imidazolinone herbicides and which are PCR positive for the transgenes.

The T1 transgenic plants were then evaluated for their improved stress tolerance according to the method described in the previous example 5. The T1 generation of single locus insertions of the the T-DNA will segregate for the transgene in a 3:1 ratio. Those progeny containing one or two copies of the transgene are tolerant of the imidazolinone herbicide, and exhibit greater tolerance of drought stress than those progeny lacking the transgenes. Tolerant plants had higher seed yields, maintained their stomatal aperture and showed only slight changes in osmotic potential and proline levels, whereas the susceptible non-transgenic control plants closed their stomata and exhibited increased osmotic potential and proline levels. Homozygous T2 plants exhibited similar phenotypes.

**Legend**

Figure 1: The glutaredoxin gene family showing the four subfamilies of glutaredoxin and thioredoxin coding sequences as determined by amino acid sequence homology.

Figure 2: Amino acid alignment of yeast and plant cDNA sequences of glutaredoxin subfamily 1 showing the presence of two conserved domains

Figure 3: Amino acid alignment of glutaredoxin subfamily 1 domain 1 across yeast and plant cDNA sequences. The amino acid position at the start of the alignment is shown in parenthesis.

Figure 4: Amino acid alignment of Glutaredoxin subfamily 1 domain 2 across yeast and plant cDNA sequences. The amino acid position at the start of the alignment is shown in parenthesis.

Figure 5: Amino acid alignments of yeast and plant cDNA sequences of glutaredoxin subfamily 2 showing the presence of two conserved domains.

Figure 6: Amino acid alignment of glutaredoxin subfamily 2 domain 1 across yeast and plant cDNA sequences.

Figure 7: Amino acid alignment of Glutaredoxin subfamily 2 domain 2 across yeast and plant cDNA sequences.

Figure 8: Amino acid alignments of yeast and plant cDNA sequences of glutaredoxin subfamily 3 showing the presence of two conserved domains.

Figure 9: Amino acid alignment of glutaredoxin subfamily 3 domain 1 across yeast and plant cDNA sequences.

Figure 10: Amino acid alignment of Glutaredoxin subfamily 3 domain 2 across yeast and plant cDNA sequences.

Figure 11: Amino acid alignments of yeast and plant cDNA sequences of glutaredoxin subfamily 4 showing the presence of two conserved domains.

Figure 12: Amino acid alignment of glutaredoxin subfamily 4 domain 1 across yeast and plant cDNA sequences.

Figure 13: Amino acid alignment of glutaredoxin subfamily 4 domain 2 across yeast and plant cDNA sequences.

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**CLAIMS**

1. A transgenic plant cell transformed by a Oxidoreductase Stress-Related Protein (ORSRP)  
5 coding nucleic acid, wherein expression of said nucleic acid in the plant cell results in increased tolerance to an environmental stress as compared to a corresponding non-transformed wild type plant cell.
2. The transgenic plant cell of claim 1, wherein the ORSRP is heat-stable.
- 10 3. The transgenic plant cell of claim 1 or 2, wherein the ORSRP is selected from yeast or plants.
4. The transgenic plant cell of claims 1 – 3, wherein the ORSRP is selected from the group  
15 comprising glutaredoxin and/or thioredoxin protein.
5. The transgenic plant cell of claims 1 – 4, wherein the ORSRP coding nucleic acid is selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ  
20 ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof.
6. The transgenic plant cell of claims 1 – 5, wherein the ORSRP coding nucleic acid is at least about 50 % homologous to SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25,  
25 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49.
7. The transgenic plant cell of claims 1 – 6, wherein the environmental stress is selected from the group consisting of salinity, drought, temperature, metal, chemical, pathogenic and oxidative stresses, or combinations thereof.
- 30 8. The transgenic plant cell of claims 1 – 7 derived from a monocotyledonous plant.
9. The transgenic plant cell of claims 1 – 7 derived from a dicotyledonous plant.

10. The transgenic plant cell of claims 1 – 9, wherein the plant is selected from the group consisting of maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed, canola, manihot, pepper, sunflower, borage, sufflower, linseed, primrose, rapeseed, turnip rape, tagetes, solanaceous plants, potato, tobacco, eggplant, tomato, Vicia species, pea, alfalfa, coffee, cacao, tea, Salix species, oil palm, coconut, perennial grass, forage crops and Arabidopsis thaliana.
11. The transgenic plant cell of claims 1 – 7, derived from a gymnosperm plant.
12. The transgenic plant cell of claims 1 – 7 or 11, wherein the plant is selected from the group of spruce, pine and fir.
13. A transgenic plant generated from a plant cell according to claims 1 – 10 and which is a monocot or dicot plant.
14. A transgenic plant of claim 13, which is selected from the group consisting of maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed, canola, manihot, pepper, sunflower, borage, sufflower, linseed, primrose, rapeseed, turnip rape, tagetes, solanaceous plants, potato, tobacco, eggplant, tomato, Vicia species, pea, alfalfa, coffee, cacao, tea, Salix species, oil palm, coconut, perennial grass, forage crops and Arabidopsis thaliana.
15. A transgenic plant generated from a plant cell according to claims 1 – 7, 11 or 12 and which is a gymnosperm plant.
16. A transgenic plant of claim 15, which is selected from the group consisting of spruce, pine and fir.
17. A seed produced by a transgenic plant of claim 13 - 16, wherein the seed is genetically homozygous for a transgene conferring an increased tolerance to environmental stress as compared to a wild type plant.
18. A plant expression cassette comprising a ORSRP coding nucleic acid selected of a group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37,

39, 41, 43, 45, 47, 49 or parts thereof operatively linked to regulatory sequences and/or targeting sequences.

- 5 19. An expression vector comprising a ORSRP encoding nucleic acid selected of a group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 or parts thereof or a plant expression cassette of claim 18, whereby expression of the ORSRP coding nucleic acid in a host cell results in increased tolerance to environmental stress as compared to a wild type host cell.
- 10 20. An expression vector comprising a ORSRP coding nucleic acid selected of a group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 or parts thereof in an antisense orientation.
- 15 21. An isolated Oxidoreductase Stress Related Protein (ORSRP) which is selected from the group comprising SEQ ID No. 16, 18, 20, 22, 24, 44 and 50.
22. An isolated Oxidoreductase Stress Related Protein (ORSRP) of claim 17 which is heat-stable.
- 20 23. An isolated Oxidoreductase Stress Related Protein (ORSRP) of claims 21 or 22 which is selected from plant.
24. An isolated Oxidoreductase Stress Related Protein (ORSRP) of claim 21-23 wherein the ORSRP is a glutaredoxin or thioredoxin protein.
- 25 25. An isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid selected from the group comprising SEQ ID No. 15, 17, 19, 21, 23, 45 and 49.
- 30 26. An isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid of claim 25 encoding an ORSRP which is heat-stable.
27. An isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid of claims 25 or 26 encoding an ORSRP which is selected from plants.



28. An isolated Oxidoreductase Stress Related Protein (ORSRP) encoding nucleic acid of claims 25-27 wherein the ORSRP is a glutaredoxin or thioredoxin .
- 5 29. A method of producing a transgenic plant comprising an ORSRP coding nucleic acid, wherein expression of the nucleic acid in the transgenic plant results in increased tolerance to environmental stress as compared to a corresponding non-transformed wild type plant, comprising
- 10 a) transforming a plant cell with an expression vector comprising the nucleic acid,  
b) generating from the plant cell a transgenic plant with an increased tolerance to environmental stress as compared to a corresponding wild type plant.
30. The method of claim 29, wherein the used ORSRP is heat-stable.
- 15 31. The method of claims 29 or 30, wherein the ORSRP is a glutaredoxin or thioredoxin protein.
32. The method of claims 29-31, wherein the ORSRP coding nucleic acid is selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof.
- 20 33. The method of claims 29-32, wherein the ORSRP coding nucleic acid is at least about 50% homologous to SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49.
- 25 34. A method of modifying stress tolerance of a plant comprising, modifying the level of expression of an ORSRP in the plant.
35. The method of claim 34, wherein the ORSRP is heat-stable.
- 30 36. The method of claims 34 or 35, wherein the ORSRP is a glutaredoxin or thioredoxin protein.
37. The method of claims 34 – 36, wherein the ORSRP encoding nucleic acid is selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No.

15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof.

- 5 38. The method of claims 34 – 37, wherein the ORSRP coding nucleic acid is at least about 50% homologous to SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49.
- 10 39. The method of claims 34 – 38, wherein an expression vector is used according to claims 19 or 20.
40. The method of claims 34 – 39, wherein the stress tolerance is decreased.
41. The method of claims 34 – 40, wherein the plant is transgenic.
- 15 42. The method of claims 34 – 41, wherein the plant is transformed with an inducible promoter that directs expression of the ORSRP.
43. The method of claims 34 – 42, wherein the promoter is tissue specific.
- 20 44. The method of claims 34 – 43, wherein the promoter is developmentally regulated.
45. The method of claims 34 – 44, wherein ORSRP expression is modified by administration of an antisense molecule and/or by double stranded RNA interference that inhibits expression of ORSPR.
- 25 46. The method of claims 34 – 45, wherein ORSRP expression is modified by administration of an targeting nucleic sequence complementary to the regulatory region of the ORSRP encoding nucleic acid and/or by a transcription factor and/or by a zinc finger protein.
- 30 47. Use of ORSRP encoding nucleic acid selected from the group comprising SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof for preparing a plant cell with increased environmental stress tolerance.

48. Use of ORSRP encoding nucleic acid selected from the group comprising SEQ ID No. SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof for preparing a plant with increased environmental stress tolerance.

5

49. Use of ORSRP encoding nucleic acid selected from the group comprising SEQ ID No. SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof or parts thereof as DNA markers for selection of plants with increased tolerance to environmental stress.

10

50. Use of ORSRP encoding nucleic acid selected from the group comprising of SEQ ID No. SEQ ID No. 1, 3, 5, 7, 9, 11, 13 of yeast and/or SEQ ID No. 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49 of plants and/or homologs thereof or parts thereof as Quantitative Trait Locus (QTL) markers for mapping genetic loci associated with environmental stress tolerance.

15

**Summary**

The present invention pertains transgenic plant cells and mature plants comprising Oxidoreductase Stress Related Proteins (ORSRP) resulting in increased tolerance and/or  
5 resistance to environmental stress as compared to non-transformed wild type cells and methods of producing such plant cells or plants. Further object of the present invention are isolated ORSRPs or ORSRP encoding nucleic acids from plants.

## Figurs

Fig 1.

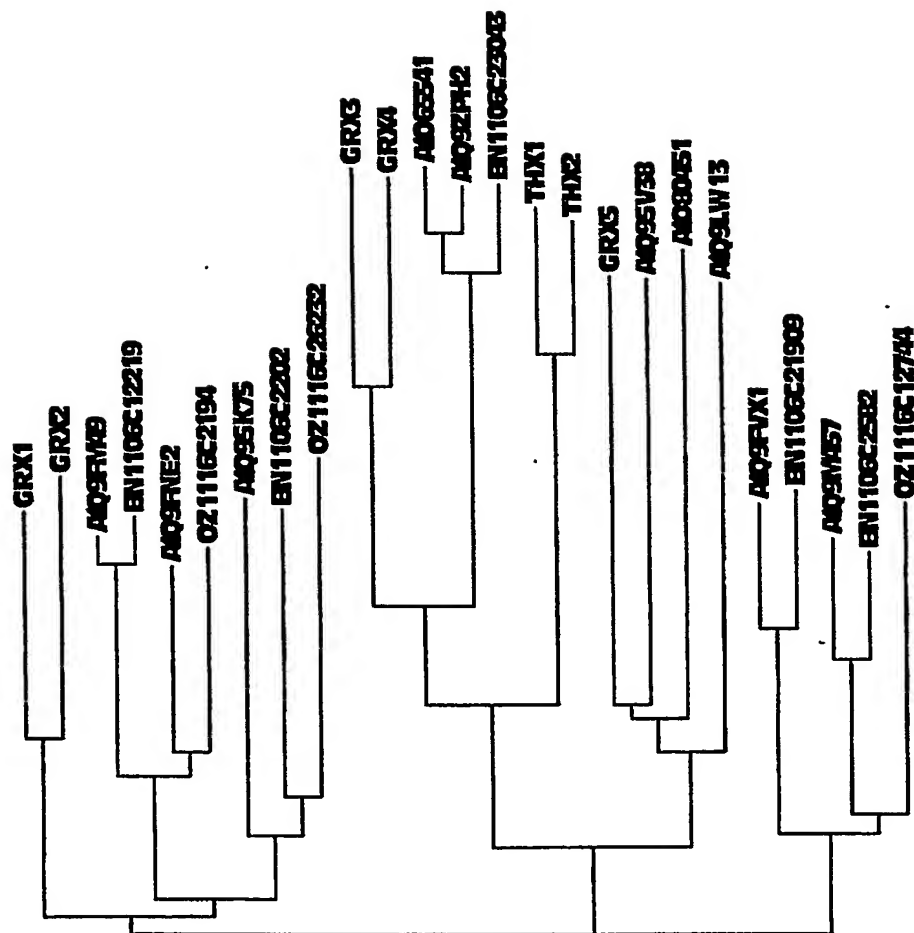


Fig. 2

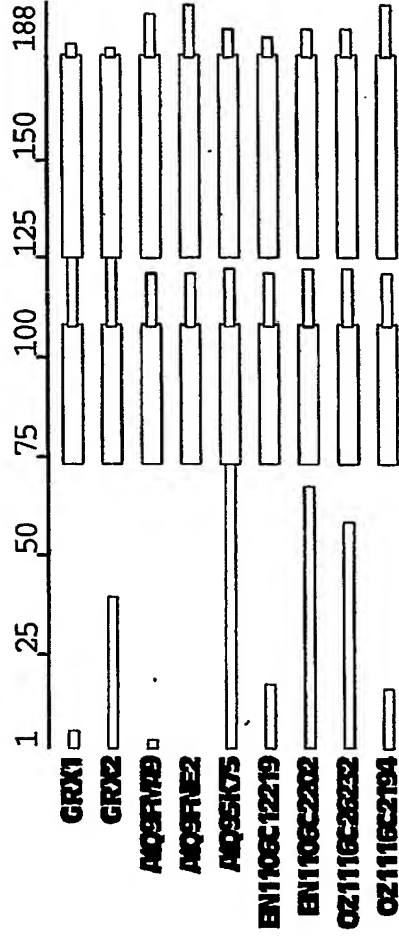


Fig. 3

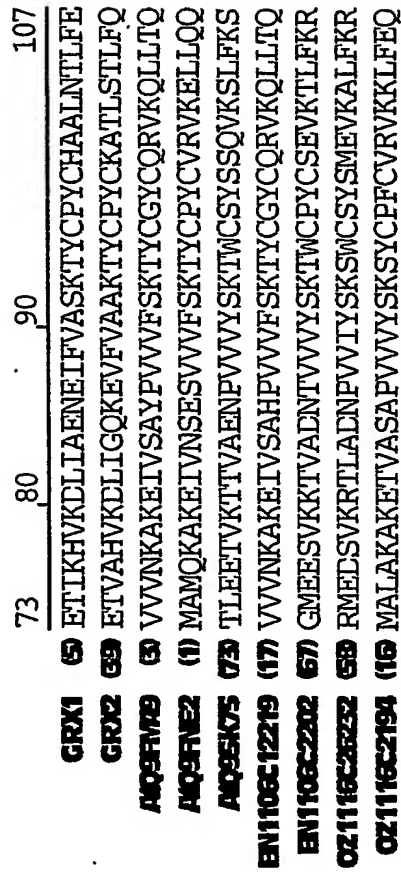


Fig. 4

	125	130	140	150	160	175			
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<b>GRX2 (91)</b>	SNGSEI	QDALE	EISGQ	KTVPN	VYING	KHIGGS	DLQEL	RETGE	LEELLEPI
<b>ATQ9FM49 (51)</b>	SDGGEI	QSAL	SEWTG	QTVPN	VFIKGN	HIGGCD	RVME	TNKQ	GKLVPLLTEA
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<b>ATQ9SK75 (122)</b>	SEGSQI	QNVLE	KITGQ	YTVPN	VFIGGK	HIGGCD	TLQ	LHNK	GELEAILAEA
<b>BN1106C12219 (65)</b>	SDGGEI	QSAL	SEWTG	QSTVP	NVFIK	GKHIGG	CDRV	MESN	KQKLVPLLTEA
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<b>OZ1116C26232 (107)</b>	AQGPQI	LQKVL	ERLTG	QSTVP	NVFIGG	KHIGG	CTDT	VKLHR	KGELEATMLSEL
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Fig. 5

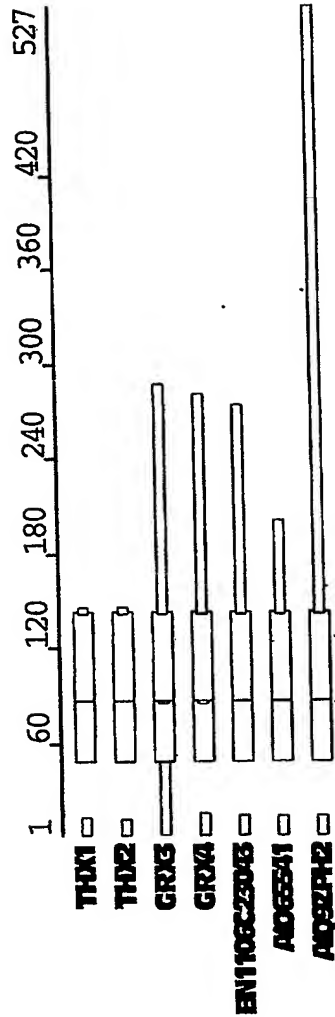


Fig. 6

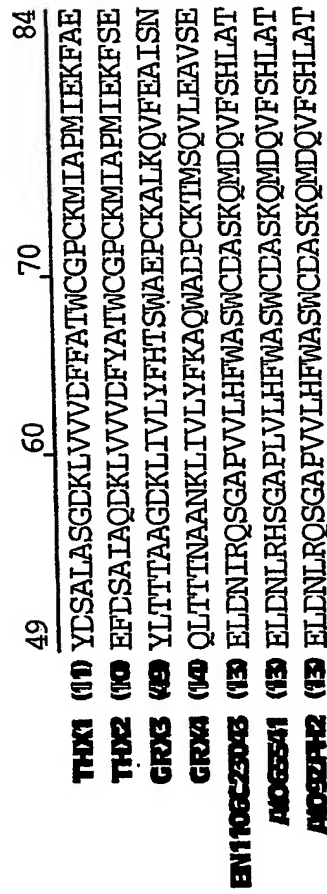




Fig. 7

	87		100		110		120		139
<b>THX1 (47)</b>	QYSDAAFYKLDVDEVS	QKAEVSSMPTL	IFYKGGKEVTR	VVGANPAAIKQA					
<b>THX2 (46)</b>	QYPQADFYKLDVDEL	GQKNEVSAMPTL	LLFKNGKEVAK	VVGANPAAIKQA					
<b>GRX3 (87)</b>	SNSNVFLSIDADENSE	ISELFEISAVPYF	IIHKGTILKEL	SGADPKEYVSL					
<b>GRX4 (52)</b>	RQEDVRFLSIDADEH	PEISDLFEIAAV	PYVFVIQNGTIV	KEISAADPKEFVKS					
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Fig. 8

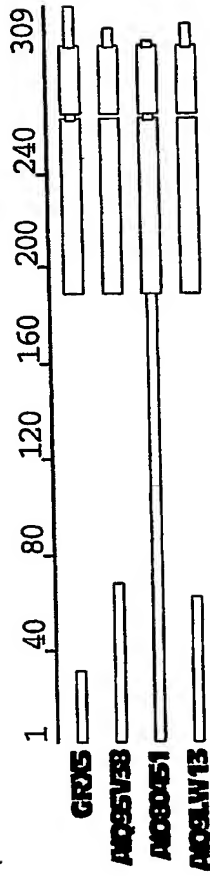


Fig. 9

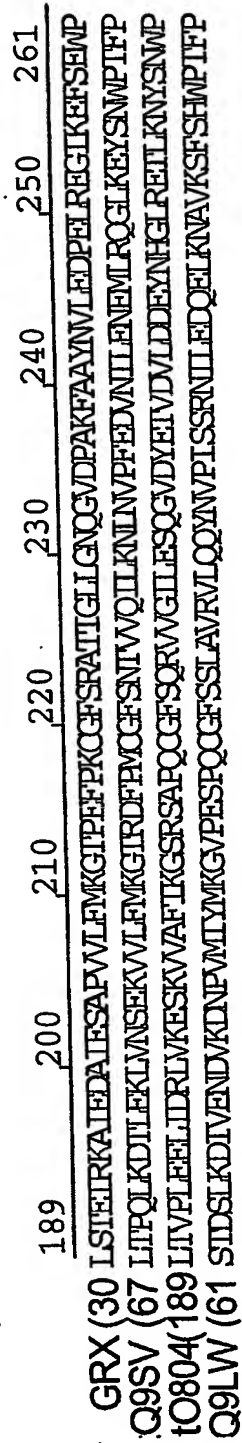


Fig. 10

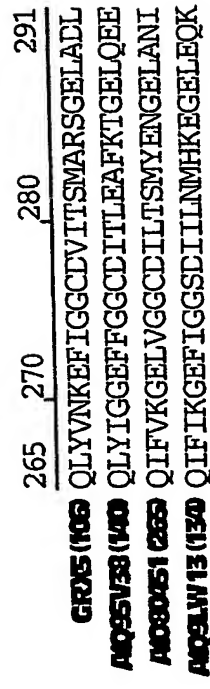


Fig. 11

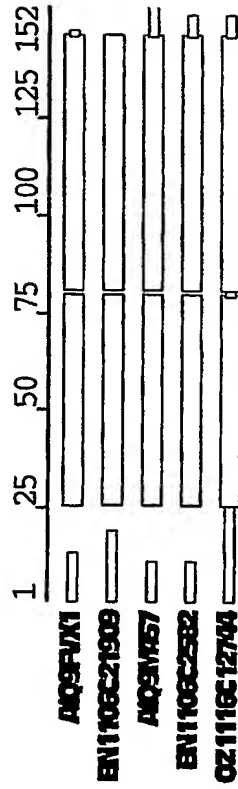


Fig. 12

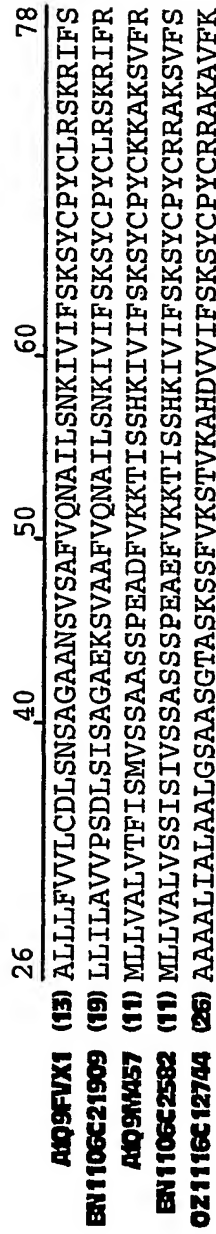
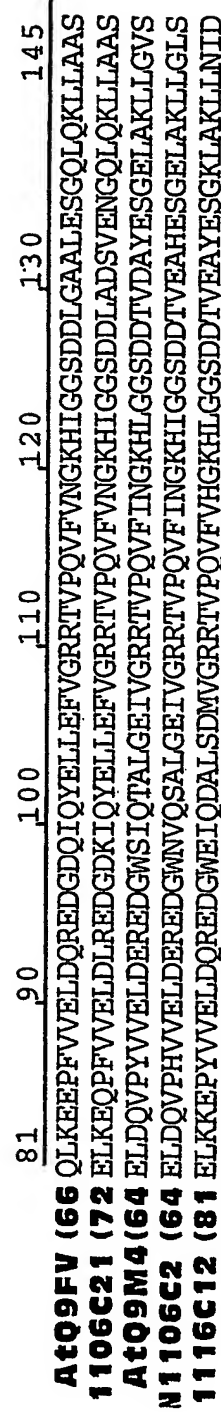


Fig. 13



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 atg gag acc aat ttt tcc ttc gac tcg aat tta att gtt att atc att 48  
 Met Glu Thr Asn Phe Ser Phe Asp Ser Asn Leu Ile Val Ile Ile Ile  
 1 5 10 15  
 atc acg ttg ttt gcc aca aga att att gct aaa aga ttt tta tct act 96  
 Ile Thr Leu Phe Ala Thr Arg Ile Ile Ala Lys Arg Phe Leu Ser Thr  
 20 25 30  
 cca aaa atg gta tcc cag gaa aca gtt gct cac gta aag gat ctg att 144  
 Pro Lys Met Val Ser Gln Glu Thr Val Ala His Val Lys Asp Leu Ile  
 35 40 45  
 ggc caa aag gaa gtg ttt gtt gca gca aag aca tac tgc cct tac tgt 192  
 Gly Gln Lys Glu Val Phe Val Ala Ala Lys Thr Tyr Cys Pro Tyr Cys  
 50 55 60  
 aaa gct act ttg tct acc ctc ttc caa gaa ttg aac gtt ccc aaa tcc 240  
 Lys Ala Thr Leu Ser Thr Leu Phe Gln Glu Leu Asn Val Pro Lys Ser  
 65 70 75 80  
 aag gcc ctt gtg ttg gaa tta gat gaa atg agc aat ggc tca gag att 288  
 Lys Ala Leu Val Leu Glu Leu Asp Glu Met Ser Asn Gly Ser Glu Ile  
 85 90 95  
 caa gac gct tta gaa gaa atc tcg ggc caa aaa act gta cct aac gta 336  
 Gln Asp Ala Leu Glu Glu Ile Ser Gly Gln Lys Thr Val Pro Asn Val  
 100 105 110  
 tac atc aat ggc aag cac att ggt ggt aac agc gat ttg gaa act ttg 384  
 Tyr Ile Asn Gly Lys His Ile Gly Gly Asn Ser Asp Leu Glu Thr Leu  
 115 120 125

aag aaa aat ggc aag tta gct gaa ata ttg aag ccg gta ttt caa tag 432  
 Lys Lys Asn Gly Lys Leu Ala Glu Ile Leu Lys Pro Val Phe Gln  
 130 135 140

<210> 4  
 <211> 143  
 <212> PRT  
 <213> *Saccharomyces cerevisiae*  
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Met Glu Thr Asn Phe Ser Phe Asp Ser Asn Leu Ile Val Ile Ile Ile  
 1 5 10 15

Ile Thr Leu Phe Ala Thr Arg Ile Ile Ala Lys Arg Phe Leu Ser Thr  
 20 25 30

Pro Lys Met Val Ser Gln Glu Thr Val Ala His Val Lys Asp Leu Ile  
 35 40 45

Gly Gln Lys Glu Val Phe Val Ala Ala Lys Thr Tyr Cys Pro Tyr Cys  
 50 55 60

Lys Ala Thr Leu Ser Thr Leu Phe Gln Glu Leu Asn Val Pro Lys Ser  
 65 70 75 80

Lys Ala Leu Val Leu Glu Leu Asp Glu Met Ser Asn Gly Ser Glu Ile  
 85 90 95

Gln Asp Ala Leu Glu Glu Ile Ser Gly Gln Lys Thr Val Pro Asn Val  
 100 105 110

Tyr Ile Asn Gly Lys His Ile Gly Gly Asn Ser Asp Leu Glu Thr Leu  
 115 120 125

Lys Lys Asn Gly Lys Leu Ala Glu Ile Leu Lys Pro Val Phe Gln  
 130 135 140

<210> 5  
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 <223> GRX3

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 Met Cys Ser Phe Gln Val Pro Ser Ala Phe Ser Phe Asn Tyr Thr Ser  
 1 5 10 15

tac tgt tat aaa cgc cac caa gca aga tat tac aca gca gca aaa ctt 96  
 Tyr Cys Tyr Lys Arg His Gln Ala Arg Tyr Tyr Thr Ala Ala Lys Leu

20	25	30	
ttt cag gaa atg cct gtt att gaa att aac gat caa gag caa ttt act			144
Phe Gln Glu Met Pro Val Ile Glu Ile Asn Asp Gln Glu Gln Phe Thr			
35	40	45	
tac cta act acc act gcg gcc ggc gac aag tta atc gtg ctt tat ttc			192
Tyr Leu Thr Thr Thr Ala Ala Gly Asp Lys Leu Ile Val Leu Tyr Phe			
50	55	60	
cat acc agt tgg gca gaa cca tgc aaa gca tta aag cag gtt ttt gag			240
His Thr Ser Trp Ala Glu Pro Cys Lys Ala Leu Lys Gln Val Phe Glu			
65	70	75	80
gcc att agt aat gag cct tcc aat tcc aac gtc tct ttc tta tcc att			288
Ala Ile Ser Asn Glu Pro Ser Asn Ser Asn Val Ser Phe Leu Ser Ile			
85	90	95	
gat gcg gac gaa aac tcg gaa att tca gaa ctt ttt gaa atc tca gct			336
Asp Ala Asp Glu Asn Ser Glu Ile Ser Glu Leu Phe Glu Ile Ser Ala			
100	105	110	
gtt cca tat ttt atc ata att cac aaa ggg aca atc tta aaa gaa tta			384
Val Pro Tyr Phe Ile Ile Ile His Lys Gly Thr Ile Leu Lys Glu Leu			
115	120	125	
tcc gcc gcg gat cca aag gag tat gtg tct tta tta gaa gac tgc aag			432
Ser Gly Ala Asp Pro Lys Glu Tyr Val Ser Leu Leu Glu Asp Cys Lys			
130	135	140	
aac tca gtc aat tcc gga tca tca caa act cat act atg gaa aat gca			480
Asn Ser Val Asn Ser Gly Ser Ser Gln Thr His Thr Met Glu Asn Ala			
145	150	155	160
aac gta aat gag ggg agt cat aat gat gaa gac gat gac gac gaa gaa			528
Asn Val Asn Glu Gly Ser His Asn Asp Glu Asp Asp Asp Asp Glu Glu			
165	170	175	
gag gaa gaa gaa act gag gag caa ata aac gct aga ttg act aaa ttg			576
Glu Glu Glu Glu Thr Glu Glu Gln Ile Asn Ala Arg Leu Thr Lys Leu			
180	185	190	
gtc aat gcc gcg ccg gta atg tta ttt atg aag ggg agc ccc tct gaa			624
Val Asn Ala Ala Pro Val Met Leu Phe Met Lys Gly Ser Pro Ser Glu			
195	200	205	
cct aaa tgc ggg ttt tcg aga caa ctt gtg ggt atc ttg aga gaa cat			672
Pro Lys Cys Gly Phe Ser Arg Gln Leu Val Gly Ile Leu Arg Glu His			
210	215	220	
caa gta aga ttt ggc ttc ttt gat ata tta aga gac gaa tct gtt aga			720
Gln Val Arg Phe Gly Phe Phe Asp Ile Leu Arg Asp Glu Ser Val Arg			
225	230	235	240
caa aac ttg aaa aag ttt tct gaa tgg cca act ttc cct caa ctt tat			768
Gln Asn Leu Lys Lys Phe Ser Glu Trp Pro Thr Phe Pro Gln Leu Tyr			
245	250	255	
ata aat ggg gag ttt caa gcc ggt tta gac att atc aag gaa tcc ttg			816
Ile Asn Gly Glu Phe Gln Gly Gly Leu Asp Ile Ile Lys Glu Ser Leu			
260	265	270	
gag gaa gac cct gat ttt ttg cag cat gct ctc caa tct taa			858



Glu Glu Asp Pro Asp Phe Leu Gln His Ala Leu Gln Ser  
 275 280 285

<210> 6  
 <211> 285  
 <212> PRT  
 <213> *Saccharomyces cerevisiae*  
 <400> 6

Met Cys Ser Phe Gln Val Pro Ser Ala Phe Ser Phe Asn Tyr Thr Ser  
 1 5 10 15

Tyr Cys Tyr Lys Arg His Gln Ala Arg Tyr Tyr Thr Ala Ala Lys Leu  
 20 25 30

Phe Gln Glu Met Pro Val Ile Glu Ile Asn Asp Gln Glu Gln Phe Thr  
 35 40 45

Tyr Leu Thr Thr Thr Ala Ala Gly Asp Lys Leu Ile Val Leu Tyr Phe  
 50 55 60

His Thr Ser Trp Ala Glu Pro Cys Lys Ala Leu Lys Gln Val Phe Glu  
 65 70 75 80

Ala Ile Ser Asn Glu Pro Ser Asn Ser Asn Val Ser Phe Leu Ser Ile  
 85 90 95

Asp Ala Asp Glu Asn Ser Glu Ile Ser Glu Leu Phe Glu Ile Ser Ala  
 100 105 110

Val Pro Tyr Phe Ile Ile Ile His Lys Gly Thr Ile Leu Lys Glu Leu  
 115 120 125

Ser Gly Ala Asp Pro Lys Glu Tyr Val Ser Leu Leu Glu Asp Cys Lys  
 130 135 140

Asn Ser Val Asn Ser Gly Ser Ser Gln Thr His Thr Met Glu Asn Ala  
 145 150 155 160

Asn Val Asn Glu Gly Ser His Asn Asp Glu Asp Asp Asp Asp Glu Glu  
 165 170 175

Glu Glu Glu Glu Thr Glu Glu Gln Ile Asn Ala Arg Leu Thr Lys Leu  
 180 185 190

Val Asn Ala Ala Pro Val Met Leu Phe Met Lys Gly Ser Pro Ser Glu  
 195 200 205

Pro Lys Cys Gly Phe Ser Arg Gln Leu Val Gly Ile Leu Arg Glu His

210	215	220	
Gln Val Arg Phe Gly Phe Phe Asp Ile Leu Arg Asp Glu Ser Val Arg			
225	230	235	240
Gln Asn Leu Lys Lys Phe Ser Glu Trp Pro Thr Phe Pro Gln Leu Tyr			
	245	250	255
Ile Asn Gly Glu Phe Gln Gly Gly Leu Asp Ile Ile Lys Glu Ser Leu			
	260	265	270
Glu Glu Asp Pro Asp Phe Leu Gln His Ala Leu Gln Ser			
	275	280	285
<210>	7		
<211>	735		
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<213>	Saccharomyces cerevisiae		
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<223>	GRX4		
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atg act gtg gtt gaa ata aaa agc cag gac caa ttt acg caa cta acc			48
Met Thr Val Val Glu Ile Lys Ser Gln Asp Gln Phe Thr Gln Leu Thr			
1	5	10	15
act aca aac gct gct aat aaa ctc att gtc tta tat ttt aaa gct caa			96
Thr Thr Asn Ala Ala Asn Lys Leu Ile Val Leu Tyr Phe Lys Ala Gln			
	20	25	30
tgg gct gat cct tgc aaa act atg agc cag gtg cta gaa gct gtt agt			144
Trp Ala Asp Pro Cys Lys Thr Met Ser Gln Val Leu Glu Ala Val Ser			
	35	40	45
gaa aaa gtt agg caa gag gat gtc cgg ttt tta tca ata gat gca gac			192
Glu Lys Val Arg Gln Glu Asp Val Arg Phe Leu Ser Ile Asp Ala Asp			
	50	55	60
gaa cat cca gaa ata tca gac ctt ttt gag att gca gcc gta cca tac			240
Glu His Pro Glu Ile Ser Asp Leu Phe Glu Ile Ala Ala Val Pro Tyr			
	65	70	75
ttc gtc ttc att caa aat ggt act att gta aaa gaa ata tca gcc gca			288
Phe Val Phe Ile Gln Asn Gly Thr Ile Val Lys Glu Ile Ser Ala Ala			
	85	90	95
gat cct aag gag ttt gtg aaa agc tta gaa att ctt tcg aat gct tct			336
Asp Pro Lys Glu Phe Val Lys Ser Leu Glu Ile Leu Ser Asn Ala Ser			
	100	105	110
gcc tca cta gcg aac aat gcc aag ggt cct aaa tct acg tct gat gag			384
Ala Ser Leu Ala Asn Asn Ala Lys Gly Pro Lys Ser Thr Ser Asp Glu			
	115	120	125
gaa agc agc ggg tct tcc gat gat gaa gag gac gaa act gaa gaa gaa			432
Glu Ser Ser Gly Ser Ser Asp Asp Glu Glu Asp Glu Thr Glu Glu Glu			

130	135	140	
ata aat gct agg ctg gtg aag cta gta caa gct gca cct gtg atg cta Ile Asn Ala Arg Leu Val Lys Leu Val Gln Ala Ala Pro Val Met Leu 145 150 155 160			480
ttc atg aaa gga agc cca tca gaa cct aaa tgc gga ttt tct aga cag Phe Met Lys Gly Ser Pro Ser Glu Pro Lys Cys Gly Phe Ser Arg Gln 165 170 175			528
tta gtt ggt atc ctc aga gaa cac caa ata agg ttc gga ttt ttt gat Leu Val Gly Ile Leu Arg Glu His Gln Ile Arg Phe Gly Phe Phe Asp 180 185 190			576
ata tta aga gac gaa aac gtt aga caa agc ttg aag aag ttt tct gat Ile Leu Arg Asp Glu Asn Val Arg Gln Ser Leu Lys Lys Phe Ser Asp 195 200 205			624
tgg cct act ttt cct cag tta tat atc aat ggg gag ttc cag gga ggt Trp Pro Thr Phe Pro Gln Leu Tyr Ile Asn Gly Glu Phe Gln Gly Gly 210 215 220			672
ttg gat att atc aag gaa tct ata gaa gaa gat cct gaa tat ttc caa Leu Asp Ile Ile Lys Glu Ser Ile Glu Glu Asp Pro Glu Tyr Phe Gln 225 230 235 240			720
cat gct cta cag taa His Ala Leu Gln			735

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 <212> PRT  
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 <400> 8

Met Thr Val Val Glu Ile Lys Ser Gln Asp Gln Phe Thr Gln Leu Thr  
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Thr Thr Asn Ala Ala Asn Lys Leu Ile Val Leu Tyr Phe Lys Ala Gln  
20 25 30

Trp Ala Asp Pro Cys Lys Thr Met Ser Gln Val Leu Glu Ala Val Ser  
35 40 45

Glu Lys Val Arg Gln Glu Asp Val Arg Phe Leu Ser Ile Asp Ala Asp  
50 55 60

Glu His Pro Glu Ile Ser Asp Leu Phe Glu Ile Ala Ala Val Pro Tyr  
65 70 75 80

Phe Val Phe Ile Gln Asn Gly Thr Ile Val Lys Glu Ile Ser Ala Ala  
85 90 95

Asp Pro Lys Glu Phe Val Lys Ser Leu Glu Ile Leu Ser Asn Ala Ser  
100 105 110

Ala Ser Leu Ala Asn Asn Ala Lys Gly Pro Lys Ser Thr Ser Asp Glu  
115 120 125

Glu Ser Ser Gly Ser Ser Asp Asp Glu Glu Asp Glu Thr Glu Glu Glu  
130 135 140

Ile Asn Ala Arg Leu Val Lys Leu Val Gln Ala Ala Pro Val Met Leu  
145 150 155 160

Phe Met Lys Gly Ser Pro Ser Glu Pro Lys Cys Gly Phe Ser Arg Gln  
165 170 175

Leu Val Gly Ile Leu Arg Glu His Gln Ile Arg Phe Gly Phe Phe Asp  
180 185 190

Ile Leu Arg Asp Glu Asn Val Arg Gln Ser Leu Lys Lys Phe Ser Asp  
195 200 205

Trp Pro Thr Phe Pro Gln Leu Tyr Ile Asn Gly Glu Phe Gln Gly Gly  
210 215 220

Leu Asp Ile Ile Lys Glu Ser Ile Glu Glu Asp Pro Glu Tyr Phe Gln  
225 230 235 240

His Ala Leu Gln

<210> 9  
<211> 453  
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<213> *Saccharomyces cerevisiae*  
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<223> GRX5

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Met Phe Leu Pro Lys Phe Asn Pro Ile Arg Ser Phe Ser Pro Ile Leu  
1 5 10 15

cgg gct aag act ctt ctt cgt tac caa aat cgg atg tat ttg agc aca 96  
Arg Ala Lys Thr Leu Leu Arg Tyr Gln Asn Arg Met Tyr Leu Ser Thr  
20 25 30

gag ata aga aaa gct att gaa gat gcc atc gaa tcg gct cca gtg gtt 144  
Glu Ile Arg Lys Ala Ile Glu Asp Ala Ile Glu Ser Ala Pro Val Val  
35 40 45

ctt ttc atg aaa ggt act cct gaa ttt ccc aag tgt gga ttt tca aga 192  
Leu Phe Met Lys Gly Thr Pro Glu Phe Pro Lys Cys Gly Phe Ser Arg  
50 55 60

gca acc att gga tta tta gga aat caa ggc gtt gac ccg gcc aaa ttt 240  
 Ala Thr Ile Gly Leu Leu Gly Asn Gln Gly Val Asp Pro Ala Lys Phe  
 65 70 75 80

gcg gct tat aat gtt tta gaa gac cca gag cta cgt gaa ggt atc aaa 288  
 Ala Ala Tyr Asn Val Leu Glu Asp Pro Glu Leu Arg Glu Gly Ile Lys  
 85 90 95

gag ttt tca gaa tgg cca act att cca cag tta tat gta aac aaa gaa 336  
 Glu Phe Ser Glu Trp Pro Thr Ile Pro Gln Leu Tyr Val Asn Lys Glu  
 100 105 110

ttc att ggt gga tgt gat gtt att aca agt atg gca cgc tct ggt gaa 384  
 Phe Ile Gly Gly Cys Asp Val Ile Thr Ser Met Ala Arg Ser Gly Glu  
 115 120 125

ttg gcc gat ttg cta gaa gag gca cag gca ttg gta cct gaa gaa gaa 432  
 Leu Ala Asp Leu Leu Glu Glu Ala Gln Ala Leu Val Pro Glu Glu Glu  
 130 135 140

gaa gaa acc aaa gat cgt tga 453  
 Glu Glu Thr Lys Asp Arg  
 145 150

<210> 10  
 <211> 150  
 <212> PRT  
 <213> *Saccharomyces cerevisiae*  
 <400> 10

Met Phe Leu Pro Lys Phe Asn Pro Ile Arg Ser Phe Ser Pro Ile Leu  
 1 5 10 15

Arg Ala Lys Thr Leu Leu Arg Tyr Gln Asn Arg Met Tyr Leu Ser Thr  
 20 25 30

Glu Ile Arg Lys Ala Ile Glu Asp Ala Ile Glu Ser Ala Pro Val Val  
 35 40 45

Leu Phe Met Lys Gly Thr Pro Glu Phe Pro Lys Cys Gly Phe Ser Arg  
 50 55 60

Ala Thr Ile Gly Leu Leu Gly Asn Gln Gly Val Asp Pro Ala Lys Phe  
 65 70 75 80

Ala Ala Tyr Asn Val Leu Glu Asp Pro Glu Leu Arg Glu Gly Ile Lys  
 85 90 95

Glu Phe Ser Glu Trp Pro Thr Ile Pro Gln Leu Tyr Val Asn Lys Glu  
 100 105 110

Phe Ile Gly Gly Cys Asp Val Ile Thr Ser Met Ala Arg Ser Gly Glu  
 115 120 125

Leu Ala Asp Leu Leu Glu Glu Ala Gln Ala Leu Val Pro Glu Glu Glu  
 130 135 140

Glu Glu Thr Lys Asp Arg  
 145 150

<210> 11  
 <211> 539  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <220>  
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 <222> (1)..(312)  
 <223> THX1

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 1 5 10 15  
 tct ggc gac aag tta gtc gtt gtt gac ttt ttt gcc aca tgg tgt ggg 96  
 Ser Gly Asp Lys Leu Val Val Val Asp Phe Phe Ala Thr Trp Cys Gly  
 20 25 30  
 cca tgt aaa atg att gca cca atg att gaa aag ttt gca gaa caa tat 144  
 Pro Cys Lys Met Ile Ala Pro Met Ile Glu Lys Phe Ala Glu Gln Tyr  
 35 40 45  
 tct gac gct gct ttt tac aag ttg gat gtt gat gaa gtc tca gat gtt 192  
 Ser Asp Ala Ala Phe Tyr Lys Leu Asp Val Asp Glu Val Ser Asp Val  
 50 55 60  
 gct caa aaa gct gaa gtt tct tcc atg cct acc cta atc ttc tac aag 240  
 Ala Gln Lys Ala Glu Val Ser Ser Met Pro Thr Leu Ile Phe Tyr Lys  
 65 70 75 80  
 ggc ggt aag gag gtt acc aga gtc gtc ggt gcc aac cca gct gct atc 288  
 Gly Gly Lys Glu Val Thr Arg Val Val Gly Ala Asn Pro Ala Ala Ile  
 85 90 95  
 aag caa gct att gct tcc aac gta tagttgccgg tatattaacg ctacgtaaag 342  
 Lys Gln Ala Ile Ala Ser Asn Val  
 100  
 tacatcatgt ttaccagttt aaataaaca ttttaaaaag aaactctatt acatctatct 402  
 atcattatgt tcttcattgt ctattgtata tttcatcatc ggtgtaacca agaattgata 462  
 aaatgtcagt catgctcttg gtattcaact tacaagggtgc agctttctgc acctttggct 522  
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<210> 12  
 <211> 104  
 <212> PRT  
 <213> *Saccharomyces cerevisiae*  
 <400> 12

Met Val Thr Gln Leu Lys Ser Ala Ser Glu Tyr Asp Ser Ala Leu Ala

1                      5                      10                      15  
 Ser Gly Asp Lys Leu Val Val Val Asp Phe Phe Ala Thr Trp Cys Gly  
                     20                      25                      30  
 Pro Cys Lys Met Ile Ala Pro Met Ile Glu Lys Phe Ala Glu Gln Tyr  
                     35                      40                      45  
 Ser Asp Ala Ala Phe Tyr Lys Leu Asp Val Asp Glu Val Ser Asp Val  
                     50                      55                      60  
 Ala Gln Lys Ala Glu Val Ser Ser Met Pro Thr Leu Ile Phe Tyr Lys  
                     65                      70                      75                      80  
 Gly Gly Lys Glu Val Thr Arg Val Val Gly Ala Asn Pro Ala Ala Ile  
                     85                      90                      95  
 Lys Gln Ala Ile Ala Ser Asn Val  
                     100

<210> 13  
 <211> 313  
 <212> DNA  
 <213> *Saccharomyces cerevisiae*  
 <220>  
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 <222> (1)..(309)  
 <223> THX2

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 Met Val Thr Gln Phe Lys Thr Ala Ser Glu Phe Asp Ser Ala Ile Ala  
 1                      5                      10                      15  
 caa gac aag cta gtt gtc gta gat ttc tac gcc act tgg tgc ggt cca 96  
 Gln Asp Lys Leu Val Val Val Asp Phe Tyr Ala Thr Trp Cys Gly Pro  
                     20                      25                      30  
 tgt aaa atg att gct cca atg att gaa aaa ttc tct gaa caa tac cca 144  
 Cys Lys Met Ile Ala Pro Met Ile Glu Lys Phe Ser Glu Gln Tyr Pro  
                     35                      40                      45  
 caa gct gat ttc tat aaa ttg gat gtc gat gaa ttg ggt gat gtt gca 192  
 Gln Ala Asp Phe Tyr Lys Leu Asp Val Asp Glu Leu Gly Asp Val Ala  
                     50                      55                      60  
 caa aag aat gaa gtt tcc gct atg cca act ttg ctt cta ttc aag aac 240  
 Gln Lys Asn Glu Val Ser Ala Met Pro Thr Leu Leu Leu Phe Lys Asn  
                     65                      70                      75                      80  
 ggt aag gaa gtt gca aag gtt gtt ggt gcc aac cca gcg gct att aag 288  
 Gly Lys Glu Val Ala Lys Val Val Gly Ala Asn Pro Ala Ala Ile Lys  
                     85                      90                      95  
 caa gcc att gct gct aat gct taaa 313  
 Gln Ala Ile Ala Ala Asn Ala

100

<210> 14  
 <211> 103  
 <212> PRT  
 <213> *Saccharomyces cerevisiae*  
 <400> 14

Met Val Thr Gln Phe Lys Thr Ala Ser Glu Phe Asp Ser Ala Ile Ala  
 1 5 10 15

Gln Asp Lys Leu Val Val Val Asp Phe Tyr Ala Thr Trp Cys Gly Pro  
 20 25 30

Cys Lys Met Ile Ala Pro Met Ile Glu Lys Phe Ser Glu Gln Tyr Pro  
 35 40 45

Gln Ala Asp Phe Tyr Lys Leu Asp Val Asp Glu Leu Gly Asp Val Ala  
 50 55 60

Gln Lys Asn Glu Val Ser Ala Met Pro Thr Leu Leu Leu Phe Lys Asn  
 65 70 75 80

Gly Lys Glu Val Ala Lys Val Val Gly Ala Asn Pro Ala Ala Ile Lys  
 85 90 95

Gln Ala Ile Ala Ala Asn Ala  
 100

<210> 15  
 <211> 657  
 <212> DNA  
 <213> *Brassica napus*  
 <220>  
 <221> CDS  
 <222> (126)..(485)  
 <223> BN1106C12219

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 aatctatagg agttgttctg tccgattcct tcaaagaata tctactgttt aggtaagagg 120  
 aagag atg ggt tct atg ttc agt gga aat cga ttg aac aag gaa gag atg 170  
 Met Gly Ser Met Phe Ser Gly Asn Arg Leu Asn Lys Glu Glu Met  
 1 5 10 15  
 gag gtt gtc gtg aac aag gcc aaa gag atc gtc tcc gct cac ccg gtc 218  
 Glu Val Val Val Asn Lys Ala Lys Glu Ile Val Ser Ala His Pro Val  
 20 25 30  
 gtt gtc ttc agc aag act tac tgt ggt tat tgc cag agg gtg aaa cag 266  
 Val Val Phe Ser Lys Thr Tyr Cys Gly Tyr Cys Gln Arg Val Lys Gln  
 35 40 45



ttg ttg aca cag cta ggt gca act ttt aaa gta ctt gag ctc gat gag 314  
Leu Leu Thr Gln Leu Gly Ala Thr Phe Lys Val Leu Glu Leu Asp Glu  
50 55 60

atg agt gat gga ggt gag atc caa tca gct tta tct gag tgg act gga 362  
Met Ser Asp Gly Gly Glu Ile Gln Ser Ala Leu Ser Glu Trp Thr Gly  
65 70 75

cag agc act gtt cct aat gtt ttc atc aaa ggc aaa cat atc ggt gga 410  
Gln Ser Thr Val Pro Asn Val Phe Ile Lys Gly Lys His Ile Gly Gly  
80 85 90 95

tgc	gat	aga	gtg	atg	gag	agt	aac	aag	caa	ggc	aag	ctt	gtg	cct	cta	458
Cys	Asp	Arg	Val	Met	Glu	Ser	Asn	Lys	Gln	Gly	Lys	Leu	Val	Pro	Leu	
			100						105					110		

ctt act gaa gct ggt gct atc tcc aat taactcttcc cagcttgagt 505  
 Leu Thr Glu Ala Gly Ala Ile Ser Asn  
 115 120

gaaaactctg aaactataaa cagtggaaat gaagaagaat gttatatgtt acatactgtc 565

aagtacccaa ataaggaaag atacttgtgg ttttcacttt gctttaaaca aaacattaac 625

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<210> 16

<211> 120

<212> PRT

<213> Brassica napus

**<400> 16**

Met Gly Ser Met Phe Ser Gly Asn Arg Leu Asn Lys Glu Glu Met Glu  
1 5 10 15

Val Val Val Asn Lys Ala Lys Glu Ile Val Ser Ala His Pro Val Val  
20 25 30

Val Phe Ser Lys Thr Tyr Cys Gly Tyr Cys Gln Arg Val Lys Gln Leu  
35 40 45

Leu Thr Gln Leu Gly Ala Thr Phe Lys Val Leu Glu Leu Asp Glu Met  
50 55 60

Ser Asp Gly Gly Glu Ile Gln Ser Ala Leu Ser Glu Trp Thr Gly Gln  
65 70 75 80

Ser Thr Val Pro Asn Val Phe Ile Lys Gly Lys His Ile Gly Gly Cys  
85 90 95

Asp Arg Val Met Glu Ser Asn Lys Gln Gly Lys Leu Val Pro Leu Leu  
 . 100 105 110

Thr Glu Ala Gly Ala Ile Ser Asn  
115 120

His Ile Leu Leu Ile Leu Ala Val Val Pro Ser Asp Leu Ser Ile Ser  
20 25 30

Ala Gly Ala Glu Lys Ser Val Ala Ala Phe Val Gln Asn Ala Ile Leu  
 35 40 45

Ser Asn Lys Ile Val Ile Phe Ser Lys Ser Tyr Cys Pro Tyr Cys Leu  
 50 55 60

Arg Ser Lys Arg Ile Phe Arg Glu Leu Lys Glu Gln Pro Phe Val Val  
 65 70 75 80

Glu Leu Asp Leu Arg Glu Asp Gly Asp Lys Ile Gln Tyr Glu Leu Leu  
 85 90 95

Glu Phe Val Gly Arg Arg Thr Val Pro Gln Val Phe Val Asn Gly Lys  
 100 105 110

His Ile Gly Gly Ser Asp Asp Leu Ala Asp Ser Val Glu Asn Gly Gln  
 115 120 125

Leu Gln Lys Leu Leu Ala Ala Ser  
 130 135

<210> 19  
 <211> 672  
 <212> DNA  
 <213> Brassica napus  
 <220>  
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 <222> (22)..(540)  
 <223> BN1106C2202

<400> 19  
 cctaagggtc agaaaatagc c atg gca gtc aca gct ttc aac cca ctg aag 51  
 Met Ala Val Thr Ala Phe Asn Pro Leu Lys  
 1 5 10  
 ctt gca tct tcg cct cga gat tcg ttt cct tca atc tcc tct tca act 99  
 Leu Ala Ser Ser Pro Arg Asp Ser Phe Pro Ser Ile Ser Ser Ser Thr  
 15 20 25  
 tct tat tcg gtg tct ctg ata agc ttc ggt ttc aga aac tcc gtc gga 147  
 Ser Tyr Ser Val Ser Leu Ile Ser Phe Gly Phe Arg Asn Ser Val Gly  
 30 35 40  
 tct cct ctc aag aaa tgt tct cta aag cag acg tgt tct gtt cga gcc 195  
 Ser Pro Leu Lys Lys Cys Ser Leu Lys Gln Thr Cys Ser Val Arg Ala  
 45 50 55  
 atg tct tct tcg tca ttc gaa tcg ggg atg gag gag agc gtg aag aaa 243  
 Met Ser Ser Ser Ser Phe Glu Ser Gly Met Glu Glu Ser Val Lys Lys  
 60 65 70  
 acg gtg gct gat aac aca gtc gtt gtt tac tcg aaa act tgg tgc cca 291  
 Thr Val Ala Asp Asn Thr Val Val Val Tyr Ser Lys Thr Trp Cys Pro  
 75 80 85 90

tac tgt tct gaa gtg aag aca ttg ttc aag aga ctt ggt gtt cag cca 339  
 Tyr Cys Ser Glu Val Lys Thr Leu Phe Lys Arg Leu Gly Val Gln Pro  
 95 100 105

ctg gtg gtt gag ttg gat gaa ctt ggt cca caa ggg aca caa cta cag 387  
 Leu Val Val Glu Leu Asp Glu Leu Gly Pro Gln Gly Thr Gln Leu Gln  
 110 115 120

aag gta ctg gaa aca ctt act ggg caa cgc act gtt cct aat gtg ttc 435  
 Lys Val Leu Glu Thr Leu Thr Gly Gln Arg Thr Val Pro Asn Val Phe  
 125 130 135

gtc gga ggc aag cac att ggt ggc tgc aca gat aca gta aac ctg aac 483  
 Val Gly Gly Lys His Ile Gly Gly Cys Thr Asp Thr Val Asn Leu Asn  
 140 145 150

agg aaa gga gaa ctg gaa ttg atg tta gct gaa gcc aac gct aaa acc 531  
 Arg Lys Gly Glu Leu Glu Leu Met Leu Ala Glu Ala Asn Ala Lys Thr  
 155 160 165 170

gat cag act tgaggaaatg atggaaactg gctttggaga tgaaccact 580  
 Asp Gln Thr

tctctctctc tctcttttgt aaacattgaa cctcgatttc tctctctaca ctttctagaa 640  
 catcattcaa ataatacatg aacagaggta aa 672

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 <212> PRT  
 <213> Brassica napus  
 <400> 20

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 1 5 10 15

Asp Ser Phe Pro Ser Ile Ser Ser Ser Thr Ser Tyr Ser Val Ser Leu  
 20 25 30

Ile Ser Phe Gly Phe Arg Asn Ser Val Gly Ser Pro Leu Lys Lys Cys  
 35 40 45

Ser Leu Lys Gln Thr Cys Ser Val Arg Ala Met Ser Ser Ser Ser Phe  
 50 55 60

Glu Ser Gly Met Glu Glu Ser Val Lys Lys Thr Val Ala Asp Asn Thr  
 65 70 75 80

Val Val Val Tyr Ser Lys Thr Trp Cys Pro Tyr Cys Ser Glu Val Lys  
 85 90 95

Thr Leu Phe Lys Arg Leu Gly Val Gln Pro Leu Val Val Glu Leu Asp  
 100 105 110

Glu Leu Gly Pro Gln Gly Thr Gln Leu Gln Lys Val Leu Glu Thr Leu  
 115 120 125

Thr Gly Gln Arg Thr Val Pro Asn Val Phe Val Gly Gly Lys His Ile  
 130 135 140

Gly Gly Cys Thr Asp Thr Val Asn Leu Asn Arg Lys Gly Glu Leu Glu  
 145 150 155 160

Leu Met Leu Ala Glu Ala Asn Ala Lys Thr Asp Gln Thr  
 165 170

<210> 21  
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 <212> DNA  
 <213> Brassica napus  
 <220>  
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 <222> (10)..(411)  
 <223> BN1106C2582

<400> 21  
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 Met Thr Met Met Arg Ser Phe Ser Met Ala Met Leu Leu Val  
 1 5 10

gca cta gtt tca tcc atc tct att gtt tct tcg gct tct tca tcc cct 99  
 Ala Leu Val Ser Ser Ile Ser Ile Val Ser Ser Ala Ser Ser Ser Pro  
 15 20 25 30

gaa gcc gag ttt gtt aag aag acc atc tct tcc cac aag atc gtt atc 147  
 Glu Ala Glu Phe Val Lys Lys Thr Ile Ser Ser His Lys Ile Val Ile  
 35 40 45

ttc tcc aaa tcc tac tgc ccg tat tgc agg aga gcc aaa tct gtg ttc 195  
 Phe Ser Lys Ser Tyr Cys Pro Tyr Cys Arg Arg Ala Lys Ser Val Phe  
 50 55 60

agt gag ctg gat cag gtt cct cat gtt gtg gag ctt gat gaa aga gaa 243  
 Ser Glu Leu Asp Gln Val Pro His Val Val Glu Leu Asp Glu Arg Glu  
 65 70 75

gat ggg tgg aac gtt cag agt gca ctt gga gag att gtt gga agg cga 291  
 Asp Gly Trp Asn Val Gln Ser Ala Leu Gly Glu Ile Val Gly Arg Arg  
 80 85 90

aca gta cca cag gtt ttc att aac gga aag cac att gga gga tca gac 339  
 Thr Val Pro Gln Val Phe Ile Asn Gly Lys His Ile Gly Gly Ser Asp  
 95 100 105 110

gat act gta gaa gcg cat gaa agc ggt gaa ctg gcc aag ctt ctc ggt 387  
 Asp Thr Val Glu Ala His Glu Ser Gly Glu Leu Ala Lys Leu Leu Gly  
 115 120 125

ctt tcc acc aaa gct gaa ctc tag gttcaatgta gttgtagttg gaggatatt 441  
 Leu Ser Thr Lys Ala Glu Leu  
 130

cagggtgaag cacttccatt ttccagtttt atgataactt gtaatgtgtt ctgaagggtta 501  
 taaacgtctt gtcatagctt tgtgaaacga tattaaaggc tacgagttgg attgagattc 561  
 aaatctgggc atgcttcaag cgaaaaaaaa aaaaacgaaa tcgtcgctct agagattccg 621  
 gggcgg 627

<210> 22  
 <211> 133  
 <212> PRT  
 <213> Brassica napus  
 <400> 22

Met Thr Met Met Arg Ser Phe Ser Met Ala Met Leu Leu Val Ala Leu  
 1 5 10 15

Val Ser Ser Ile Ser Ile Val Ser Ser Ala Ser Ser Ser Pro Glu Ala  
 20 25 30

Glu Phe Val Lys Lys Thr Ile Ser Ser His Lys Ile Val Ile Phe Ser  
 35 40 45

Lys Ser Tyr Cys Pro Tyr Cys Arg Arg Ala Lys Ser Val Phe Ser Glu  
 50 55 60

Leu Asp Gln Val Pro His Val Val Glu Leu Asp Glu Arg Glu Asp Gly  
 65 70 75 80

Trp Asn Val Gln Ser Ala Leu Gly Glu Ile Val Gly Arg Arg Thr Val  
 85 90 95

Pro Gln Val Phe Ile Asn Gly Lys His Ile Gly Gly Ser Asp Asp Thr  
 100 105 110

Val Glu Ala His Glu Ser Gly Glu Leu Ala Lys Leu Leu Gly Leu Ser  
 115 120 125

Thr Lys Ala Glu Leu  
 130

<210> 23  
 <211> 743  
 <212> DNA  
 <213> Brassica napus  
 <220>  
 <221> CDS  
 <222> (42) .. (680)  
 <223> BN1106C23043

<400> 23  
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 Met Gly Gly Ala Val

	1	5	
aag gat att gct tca aag tcc gag ctt gat aac att cgc cag agc ggc Lys Asp Ile Ala Ser Lys Ser Glu Leu Asp Asn Ile Arg Gln Ser Gly	10	20	104
gca ccg gtg gtg ctt cac ttc tgg gct tcg tgg tgt gat gct tcg aag Ala Pro Val Val Leu His Phe Trp Ala Ser Trp Cys Asp Ala Ser Lys	25	30	152
cag atg gat caa gtc ttc tct cac ctc gct acc gac ttc cct cgc gcc Gln Met Asp Gln Val Phe Ser His Leu Ala Thr Asp Phe Pro Arg Ala	40	45	200
cac ttc ttt agg gta gaa gct gag gaa cat cct gag ata tct gaa gct His Phe Phe Arg Val Glu Ala Glu Glu His Pro Glu Ile Ser Glu Ala	55	60	248
tac tct gtt tct gct gtt ccc tat ttc gtc ttc ttc aag gat ggc aaa Tyr Ser Val Ser Ala Val Pro Tyr Phe Val Phe Phe Lys Asp Gly Lys	70	75	296
gct gtg gat aca ctt gag gga gca gat cca tca agt tta gcc aat aaa Ala Val Asp Thr Leu Glu Gly Ala Asp Pro Ser Ser Leu Ala Asn Lys	90	95	344
gtt ggc aaa gtc gct ggt tcc agc act tct gct gag cct gct gct cct Val Gly Lys Val Ala Gly Ser Ser Thr Ser Ala Glu Pro Ala Ala Pro	105	110	392
gca agc cta ggg ctg gct gca ggg cca acg att ctc gaa acc gtc aag Ala Ser Leu Gly Leu Ala Ala Gly Pro Thr Ile Leu Glu Thr Val Lys	120	125	440
gag aat gcg aaa gct act tcg aaa gac cga gct cag cct tta tcc tcc Glu Asn Ala Lys Ala Thr Ser Lys Asp Arg Ala Gln Pro Leu Ser Ser	135	140	488
acc acc aag gaa gct ctc aat acc cgt ttg gag aaa ctc acc aac tct Thr Thr Lys Glu Ala Leu Asn Thr Arg Leu Glu Lys Leu Thr Asn Ser	150	155	536
cac cct gtt atg ttg ttc atg aaa ggt acc cct gag gag cct atg tgc His Pro Val Met Leu Phe Met Lys Gly Thr Pro Glu Glu Pro Met Cys	170	175	584
ggt ttc agc aag aac gta gtt aac atc ttg aag gag gag gaa gtt gag Gly Phe Ser Lys Asn Val Val Asn Ile Leu Lys Glu Glu Glu Val Glu	185	190	632
ttc gga agt ttc gat ata ctt tcg gac aat gaa gtc cgt gaa ggt ctg Phe Gly Ser Phe Asp Ile Leu Ser Asp Asn Glu Val Arg Glu Gly Leu	200	205	680
aagaagttct tcaactggcc aacgtaccct cagctgtaca gcatcggaga gctactctgt			740
gga			743

&lt;210&gt; 24

&lt;211&gt; 213

&lt;212&gt; PRT

&lt;213&gt; Brassica napus

&lt;400&gt; 24

Met Gly Gly Ala Val Lys Asp Ile Ala Ser Lys Ser Glu Leu Asp Asn  
1 5 10 15

Ile Arg Gln Ser Gly Ala Pro Val Val Leu His Phe Trp Ala Ser Trp  
20 25 30

Cys Asp Ala Ser Lys Gln Met Asp Gln Val Phe Ser His Leu Ala Thr  
35 40 45

Asp Phe Pro Arg Ala His Phe Phe Arg Val Glu Ala Glu Glu His Pro  
50 55 60

Glu Ile Ser Glu Ala Tyr Ser Val Ser Ala Val Pro Tyr Phe Val Phe  
65 70 75 80

Phe Lys Asp Gly Lys Ala Val Asp Thr Leu Glu Gly Ala Asp Pro Ser  
85 90 95

Ser Leu Ala Asn Lys Val Gly Lys Val Ala Gly Ser Ser Thr Ser Ala  
100 105 110

Glu Pro Ala Ala Pro Ala Ser Leu Gly Leu Ala Ala Gly Pro Thr Ile  
115 120 125

Leu Glu Thr Val Lys Glu Asn Ala Lys Ala Thr Ser Lys Asp Arg Ala  
130 135 140

Gln Pro Leu Ser Ser Thr Thr Lys Glu Ala Leu Asn Thr Arg Leu Glu  
145 150 155 160

Lys Leu Thr Asn Ser His Pro Val Met Leu Phe Met Lys Gly Thr Pro  
165 170 175

Glu Glu Pro Met Cys Gly Phe Ser Lys Asn Val Val Asn Ile Leu Lys  
180 185 190

Glu Glu Glu Val Glu Phe Gly Ser Phe Asp Ile Leu Ser Asp Asn Glu  
195 200 205

Val Arg Glu Gly Leu  
210

&lt;210&gt; 25

&lt;211&gt; 336

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

&lt;220&gt;



&lt;221&gt; CDS

&lt;222&gt; (1)..(333)

&lt;223&gt; AtQ9FM49

&lt;400&gt; 25

atg gag gtg gtg gtg aac aag gct aaa gag atc gtc tct gct tat ccc 48

Met Glu Val Val Val Asn Lys Ala Lys Glu Ile Val Ser Ala Tyr Pro

1 5 10 15

gtt gtt gtc ttc agc aag aca tac tgt ggt tat tgc cag agg gtg aag 96

Val Val Val Phe Ser Lys Thr Tyr Cys Gly Tyr Cys Gln Arg Val Lys

20 25 30

cag tta ctg acg cag cta gga gca act ttt aaa gta ctt gag ctc gat 144

Gln Leu Leu Thr Gln Leu Gly Ala Thr Phe Lys Val Leu Glu Leu Asp

35 40 45

gaa atg agt gat gga ggt gag atc caa tca gct tta tca gag tgg act 192

Glu Met Ser Asp Gly Gly Glu Ile Gln Ser Ala Leu Ser Glu Trp Thr

50 55 60

gga cag acc aca gtt cca aac gtc ttc atc aaa gga aac cac atc ggt 240

Gly Gln Thr Thr Val Pro Asn Val Phe Ile Lys Gly Asn His Ile Gly

65 70 75 80

gga tgc gat aga gtg atg gag acc aac aag caa ggc aag ctt gtg cct 288

Gly Cys Asp Arg Val Met Glu Thr Asn Lys Gln Gly Lys Leu Val Pro

85 90 95

cta ctt act gaa gct ggg gct att gca gat aac tct tct caa ctt tga 336

Leu Leu Thr Glu Ala Gly Ala Ile Ala Asp Asn Ser Ser Gln Leu

100 105 110

&lt;210&gt; 26

&lt;211&gt; 111

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 26

Met Glu Val Val Val Asn Lys Ala Lys Glu Ile Val Ser Ala Tyr Pro

1 5 10 15

Val Val Val Phe Ser Lys Thr Tyr Cys Gly Tyr Cys Gln Arg Val Lys

20 25 30

Gln Leu Leu Thr Gln Leu Gly Ala Thr Phe Lys Val Leu Glu Leu Asp

35 40 45

Glu Met Ser Asp Gly Gly Glu Ile Gln Ser Ala Leu Ser Glu Trp Thr

50 55 60

Gly Gln Thr Thr Val Pro Asn Val Phe Ile Lys Gly Asn His Ile Gly

65 70 75 80

Gly Cys Asp Arg Val Met Glu Thr Asn Lys Gln Gly Lys Leu Val Pro

85 90 95

Leu Leu Thr Glu Ala Gly Ala Ile Ala Asp Asn Ser Ser Gln Leu  
 100 105 110

<210> 27  
 <211> 336  
 <212> DNA  
 <213> Arabidopsis thaliana  
 <220>  
 <221> CDS  
 <222> (1)..(333)  
 <223> AtQ9FNE2

<400> 27  
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 Met Ala Met Gln Lys Ala Lys Glu Ile Val Asn Ser Glu Ser Val Val  
 1 5 10 15  
 gtt ttc agc aag act tat tgt cca tat tgc gtg aga gtg aag gag ctt 96  
 Val Phe Ser Lys Thr Tyr Cys Pro Tyr Cys Val Arg Val Lys Glu Leu  
 20 25 30  
 ttg caa caa ttg gga gct aag ttc aag gcc gtt gag ctc gac acc gaa 144  
 Leu Gln Gln Leu Gly Ala Lys Phe Lys Ala Val Glu Leu Asp Thr Glu  
 35 40 45  
 agt gat ggt agc caa att caa tca ggt ctc gca gaa tgg aca gga caa 192  
 Ser Asp Gly Ser Gln Ile Gln Ser Gly Leu Ala Glu Trp Thr Gly Gln  
 50 55 60  
 cgt acc gtg cct aat gtg ttt ata gga gga aat cac atc ggt ggc tgt 240  
 Arg Thr Val Pro Asn Val Phe Ile Gly Gly Asn His Ile Gly Gly Cys  
 65 70 75 80  
 gat gca aca tca aac ttg cat aaa gat ggg aag ttg gtt ccg ctg tta 288  
 Asp Ala Thr Ser Asn Leu His Lys Asp Gly Lys Leu Val Pro Leu Leu  
 85 90 95  
 act gaa gct gga gcg atc gca gga aag act gca aca act tct gct taa 336  
 Thr Glu Ala Gly Ala Ile Ala Gly Lys Thr Ala Thr Thr Ser Ala  
 100 105 110

<210> 28  
 <211> 111  
 <212> PRT  
 <213> Arabidopsis thaliana  
 <400> 28

Met Ala Met Gln Lys Ala Lys Glu Ile Val Asn Ser Glu Ser Val Val  
 1 5 10 15  
 Val Phe Ser Lys Thr Tyr Cys Pro Tyr Cys Val Arg Val Lys Glu Leu  
 20 25 30  
 Leu Gln Gln Leu Gly Ala Lys Phe Lys Ala Val Glu Leu Asp Thr Glu  
 35 40 45  
 Ser Asp Gly Ser Gln Ile Gln Ser Gly Leu Ala Glu Trp Thr Gly Gln

50

55

60

Arg Thr Val Pro Asn Val Phe Ile Gly Gly Asn His Ile Gly Gly Cys  
 65 70 75 80

Asp Ala Thr Ser Asn Leu His Lys Asp Gly Lys Leu Val Pro Leu Leu  
 85 90 95

Thr Glu Ala Gly Ala Ile Ala Gly Lys Thr Ala Thr Thr Ser Ala  
 100 105 110

<210> 29  
 <211> 393  
 <212> DNA  
 <213> Arabidopsis thaliana  
 <220>  
 <221> CDS  
 <222> (1)..(390)  
 <223> AtQ9FVX1

<400> 29

atg gtt gac cag agt cct cgc cgt gtt gtc gtg gcg gcg ctc cta ttg 48  
 Met Val Asp Gln Ser Pro Arg Arg Val Val Val Ala Ala Leu Leu Leu  
 1 5 10 15

ttt gtg gtt ctg tgc gat ctt tcg aat tct gcg gga gct gcg aat tct 96  
 Phe Val Val Leu Cys Asp Leu Ser Asn Ser Ala Gly Ala Ala Asn Ser  
 20 25 30

gtg tca gct ttc gtt cag aac gcc atc ttg tcc aac aag att gtc atc 144  
 Val Ser Ala Phe Val Gln Asn Ala Ile Leu Ser Asn Lys Ile Val Ile  
 35 40 45

ttc tcc aaa tcc tac tgc ccg tat tgt ttg cgg tcg aaa cgt ata ttc 192  
 Phe Ser Lys Ser Tyr Cys Pro Tyr Cys Leu Arg Ser Lys Arg Ile Phe  
 50 55 60

agc caa ctt aag gaa gag cca ttt gtt gtg gag ctt gat cag aga gag 240  
 Ser Gln Leu Lys Glu Glu Pro Phe Val Val Glu Leu Asp Gln Arg Glu  
 65 70 75 80

gac gga gat caa atc cag tat gag ctt tta gaa ttc gtt ggt cgt cgt 288  
 Asp Gly Asp Gln Ile Gln Tyr Glu Leu Leu Glu Phe Val Gly Arg Arg  
 85 90 95

act gtc ccg caa gtt ttt gtt aac ggc aag cat att ggt gga tca gat 336  
 Thr Val Pro Gln Val Phe Val Asn Gly Lys His Ile Gly Gly Ser Asp  
 100 105 110

gat ctt gga gct gct ttg gag agt ggt cag ttg caa aag ctt ctt gct 384  
 Asp Leu Gly Ala Ala Leu Glu Ser Gly Gln Leu Gln Lys Leu Leu Ala  
 115 120 125

gca agt tga 393  
 Ala Ser  
 130

<210> 30

<211> 130  
 <212> PRT  
 <213> Arabidopsis thaliana  
 <400> 30

Met Val Asp Gln Ser Pro Arg Arg Val Val Val Ala Ala Leu Leu Leu  
 1 5 10 15

Phe Val Val Leu Cys Asp Leu Ser Asn Ser Ala Gly Ala Ala Asn Ser  
 20 25 30

Val Ser Ala Phe Val Gln Asn Ala Ile Leu Ser Asn Lys Ile Val Ile  
 35 40 45

Phe Ser Lys Ser Tyr Cys Pro Tyr Cys Leu Arg Ser Lys Arg Ile Phe  
 50 55 60

Ser Gln Leu Lys Glu Glu Pro Phe Val Val Glu Leu Asp Gln Arg Glu  
 65 70 75 80

Asp Gly Asp Gln Ile Gln Tyr Glu Leu Leu Glu Phe Val Gly Arg Arg  
 85 90 95

Thr Val Pro Gln Val Phe Val Asn Gly Lys His Ile Gly Gly Ser Asp  
 100 105 110

Asp Leu Gly Ala Ala Leu Glu Ser Gly Gln Leu Gln Lys Leu Leu Ala  
 115 120 125

Ala Ser  
 130

<210> 31  
 <211> 629  
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 <223> AtQ9M457

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 Met Thr Met  
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ttt aga tct atc tcc atg gta atg ctg ctc gtc gca cta gtt aca ttc 104  
 Phe Arg Ser Ile Ser Met Val Met Leu Leu Val Ala Leu Val Thr Phe  
 5 10 15

att tct atg gtt tct tct gct gct tcg tcc cca gaa gcc gac ttt gtt 152  
 Ile Ser Met Val Ser Ser Ala Ala Ser Ser Pro Glu Ala Asp Phe Val  
 20 25 30 35

aag aag act atc tct tcc cat aag atc gtc att ttc tcc aaa tcc tac 200  
 Lys Lys Thr Ile Ser Ser His Lys Ile Val Ile Phe Ser Lys Ser Tyr  
 40 45 50

tgc ccc tac tgc aag aaa gct aaa tca gtg ttc aga gag ctg gat caa 248  
 Cys Pro Tyr Cys Lys Lys Ala Lys Ser Val Phe Arg Glu Leu Asp Gln  
 55 60 65

gtt cct tat gtt gtc gag ctt gat gaa aga gaa gat ggt tgg agc atc 296  
 Val Pro Tyr Val Val Glu Leu Asp Glu Arg Glu Asp Gly Trp Ser Ile  
 70 75 80

cag act gca ctt gga gag att gtt gga agg cga aca gta ccg caa gtc 344  
 Gln Thr Ala Leu Gly Glu Ile Val Gly Arg Arg Thr Val Pro Gln Val  
 85 90 95

ttc att aac gga aaa cat ctc gga gga tca gat gat acc gta gat gcg 392  
 Phe Ile Asn Gly Lys His Leu Gly Gly Ser Asp Asp Thr Val Asp Ala  
 100 105 110 115

tat gag agc ggt gaa ctc gcc aag ctt ctt ggt gtt tcc ggg aac aaa 440  
 Tyr Glu Ser Gly Glu Leu Ala Lys Leu Leu Gly Val Ser Gly Asn Lys  
 120 125 130

gaa gct gaa ctc taggttatat atagttggaa gaattgataa cactctctgt 492  
 Glu Ala Glu Leu  
 135

gatgcttagg tgtaagcaat tcaatttcca tttgtattgt gttctgcagc ttgatcatga 552

ccttgtgaca gcttgatctt gcctttttaa cgtatcttat caaagaccac attctgagtt 612

aaaaaaaaa aaaaaaa 629

<210> 32

<211> 135

<212> PRT

<213> Arabidopsis thaliana

<400> 32

Met Thr Met Phe Arg Ser Ile Ser Met Val Met Leu Leu Val Ala Leu  
 1 5 10 15

Val Thr Phe Ile Ser Met Val Ser Ser Ala Ala Ser Ser Pro Glu Ala  
 20 25 30

Asp Phe Val Lys Lys Thr Ile Ser Ser His Lys Ile Val Ile Phe Ser  
 35 40 45

Lys Ser Tyr Cys Pro Tyr Cys Lys Lys Ala Lys Ser Val Phe Arg Glu  
 50 55 60

Leu Asp Gln Val Pro Tyr Val Val Glu Leu Asp Glu Arg Glu Asp Gly  
 65 70 75 80

Trp Ser Ile Gln Thr Ala Leu Gly Glu Ile Val Gly Arg Arg Thr Val  
 85 90 95

Pro Gln Val Phe Ile Asn Gly Lys His Leu Gly Gly Ser Asp Asp Thr  
 100 105 110

Val Asp Ala Tyr Glu Ser Gly Glu Leu Ala Lys Leu Leu Gly Val Ser  
 115 120 125

Gly Asn Lys Glu Ala Glu Leu  
 130 135

<210> 33  
 <211> 540  
 <212> DNA  
 <213> Arabidopsis thaliana  
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 <222> (1)..(537)  
 <223> AtQ9SK75

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 1 5 10 15

aat tca aat cca gca atc tct ttc tcc atg tta agc gga atc aga aac 96  
 Asn Ser Asn Pro Ala Ile Ser Phe Ser Met Leu Ser Gly Ile Arg Asn  
 20 25 30

ttg ggc atg tta cct ttc agg aga tgt cta aag ccg aca gtt atc gga 144  
 Leu Gly Met Leu Pro Phe Arg Arg Cys Leu Lys Pro Thr Val Ile Gly  
 35 40 45

atc gcg tcg tgg cca cca ctc cgt tgt tct tct gtt aag gct atg tct 192  
 Ile Ala Ser Trp Pro Pro Leu Arg Cys Ser Ser Val Lys Ala Met Ser  
 50 55 60

tca tca tcg tct tcg tct gga tcg aca ttg gag gag act gtt aaa acg 240  
 Ser Ser Ser Ser Ser Ser Gly Ser Thr Leu Glu Glu Thr Val Lys Thr  
 65 70 75 80

act gtg gca gag aac cct gtc gtt gtt tac tcc aaa acc tgg tgc tca 288  
 Thr Val Ala Glu Asn Pro Val Val Val Tyr Ser Lys Thr Trp Cys Ser  
 85 90 95

tac tcg tct caa gtg aag tcc ttg ttc aag agt ctt caa gtt gag cca 336  
 Tyr Ser Ser Gln Val Lys Ser Leu Phe Lys Ser Leu Gln Val Glu Pro  
 100 105 110

ctg gtt gtt gaa ttg gat caa ctt ggt tca gaa ggg tcg cag ctg cag 384  
 Leu Val Val Glu Leu Asp Gln Leu Gly Ser Glu Gly Ser Gln Leu Gln  
 115 120 125

aat gtg ttg gag aaa att act gga caa tac act gtt ccc aat gtt ttc 432  
 Asn Val Leu Glu Lys Ile Thr Gly Gln Tyr Thr Val Pro Asn Val Phe  
 130 135 140

atc gga ggc aag cac att ggt ggc tgc tca gat aca ttg cag ctg cac 480  
 Ile Gly Gly Lys His Ile Gly Gly Cys Ser Asp Thr Leu Gln Leu His  
 145 150 155 160

aat aaa gga gag ctg gaa gca att tta gct gaa gcc aat gga aaa aac 528  
 Asn Lys Gly Glu Leu Glu Ala Ile Leu Ala Glu Ala Asn Gly Lys Asn  
                   165                                  170                                  175

ggt cag acc tag  
 Gly Gln Thr 540

<210> 34  
 <211> 179  
 <212> PRT  
 <213> Arabidopsis thaliana  
 <400> 34

Met Val Ala Ala Thr Val Asn Leu Ala Asn Met Thr Trp Thr Ser Leu  
   1                                  5                                  10                                  15

Asn Ser Asn Pro Ala Ile Ser Phe Ser Met Leu Ser Gly Ile Arg Asn  
                   20                                  25                                  30

Leu Gly Met Leu Pro Phe Arg Arg Cys Leu Lys Pro Thr Val Ile Gly  
           35                                  40                                  45

Ile Ala Ser Trp Pro Pro Leu Arg Cys Ser Ser Val Lys Ala Met Ser  
   50                                  55                                  60

Ser Ser Ser Ser Ser Ser Gly Ser Thr Leu Glu Glu Thr Val Lys Thr  
   65                                  70                                  75                                  80

Thr Val Ala Glu Asn Pro Val Val Val Tyr Ser Lys Thr Trp Cys Ser  
                   85                                  90                                  95

Tyr Ser Ser Gln Val Lys Ser Leu Phe Lys Ser Leu Gln Val Glu Pro  
                   100                                  105                                  110

Leu Val Val Glu Leu Asp Gln Leu Gly Ser Glu Gly Ser Gln Leu Gln  
           115                                  120                                  125

Asn Val Leu Glu Lys Ile Thr Gly Gln Tyr Thr Val Pro Asn Val Phe  
   130                                  135                                  140

Ile Gly Gly Lys His Ile Gly Gly Cys Ser Asp Thr Leu Gln Leu His  
   145                                  150                                  155                                  160

Asn Lys Gly Glu Leu Glu Ala Ile Leu Ala Glu Ala Asn Gly Lys Asn  
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Gly Gln Thr

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Met Ala Ala Ser Leu Ser Ser Arg Leu Ile Lys Gly Ile Ala Asn Leu	
1 5 10 15	
aaa gct gtt cgt tct agc aga ttg acg tct gca tca gtc tac caa aat	96
Lys Ala Val Arg Ser Ser Arg Leu Thr Ser Ala Ser Val Tyr Gln Asn	
20 25 30	
ggg atg atg aga ttt tcc tca aca gtg cca agt gat tca gat aca cat	144
Gly Met Met Arg Phe Ser Ser Thr Val Pro Ser Asp Ser Asp Thr His	
35 40 45	
gat gat ttc aag cct aca caa aaa gtc cct ccc gat tct acg gac tca	192
Asp Asp Phe Lys Pro Thr Gln Lys Val Pro Pro Asp Ser Thr Asp Ser	
50 55 60	
ctt aaa gat atc gtt gag aat gat gtg aag gat aat cct gtt atg atc	240
Leu Lys Asp Ile Val Glu Asn Asp Val Lys Asp Asn Pro Val Met Ile	
65 70 75 80	
tac atg aaa ggt gtc cct gaa tct cct cag tgt ggg ttt agc tca cta	288
Tyr Met Lys Gly Val Pro Glu Ser Pro Gln Cys Gly Phe Ser Ser Leu	
85 90 95	
gcc gtc aga gtt ttg cag caa tat aat gtt cct atc agt tct aga aac	336
Ala Val Arg Val Leu Gln Gln Tyr Asn Val Pro Ile Ser Ser Arg Asn	
100 105 110	
att cta gaa gac caa gag ttg aaa aac gct gtg aaa tcc ttc agc cac	384
Ile Leu Glu Asp Gln Glu Leu Lys Asn Ala Val Lys Ser Phe Ser His	
115 120 125	
tgg cct acg ttt cca cag atc ttc att aag gga gag ttc att ggc ggc	432
Trp Pro Thr Phe Pro Gln Ile Phe Ile Lys Gly Glu Phe Ile Gly Gly	
130 135 140	
tca gac atc atc ctt aac atg cac aag gaa ggt gaa ttg gag cag aag	480
Ser Asp Ile Ile Leu Asn Met His Lys Glu Gly Glu Leu Glu Gln Lys	
145 150 155 160	
ctt aaa gac gtc tcc gga aac caa gat tga	510
Leu Lys Asp Val Ser Gly Asn Gln Asp	
165	

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                     20                      25                      30  
 Gly Met Met Arg Phe Ser Ser Thr Val Pro Ser Asp Ser Asp Thr His  
                     35                      40                      45  
 Asp Asp Phe Lys Pro Thr Gln Lys Val Pro Pro Asp Ser Thr Asp Ser  
                     50                      55                      60  
 Leu Lys Asp Ile Val Glu Asn Asp Val Lys Asp Asn Pro Val Met Ile  
                     65                      70                      75                      80  
 Tyr Met Lys Gly Val Pro Glu Ser Pro Gln Cys Gly Phe Ser Ser Leu  
                     85                      90                      95  
 Ala Val Arg Val Leu Gln Gln Tyr Asn Val Pro Ile Ser Ser Arg Asn  
                     100                      105                      110  
 Ile Leu Glu Asp Gln Glu Leu Lys Asn Ala Val Lys Ser Phe Ser His  
                     115                      120                      125  
 Trp Pro Thr Phe Pro Gln Ile Phe Ile Lys Gly Glu Phe Ile Gly Gly  
                     130                      135                      140  
 Ser Asp Ile Ile Leu Asn Met His Lys Glu Gly Glu Leu Glu Gln Lys  
                     145                      150                      155                      160  
 Leu Lys Asp Val Ser Gly Asn Gln Asp  
                     165

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 gtc gtc tcc tcc tcc gtt acc aac aag cct cac tct atc aga ttc tct                      96  
 Val Val Ser Ser Ser Val Thr Asn Lys Pro His Ser Ile Arg Phe Ser  
                     20                      25                      30  
 ctt aaa cca acg tcg gca ctc gtc gtc cat aac cat cag cta tcg ttc                      144  
 Leu Lys Pro Thr Ser Ala Leu Val Val His Asn His Gln Leu Ser Phe

35	40	45	
tac ggt tcg aat ctc aag ctg aaa cca act aaa ttc cga tgc tca gcg			192
Tyr Gly Ser Asn Leu Lys	Leu Lys Pro Thr Lys	Phe Arg Cys Ser Ala	
50	55	60	
tcg gct ctt acg ccg caa ctt aaa gac acg ctg gag aaa ctg gtg aat			240
Ser Ala Leu Thr Pro Gln Leu Lys Asp Thr	Leu Glu Lys Leu Val Asn		
65	70	75 80	
tcg gag aaa gtg gtt ctg ttt atg aaa gga acg aga gac ttc ccg atg			288
Ser Glu Lys Val Val Leu Phe Met Lys Gly Thr Arg Asp Phe Pro Met			
	85	90 95	
tgt gga ttc tcc aac act gtg gtt cag att ttg aag aat ctg aat gtt			336
Cys Gly Phe Ser Asn Thr Val Val Gln Ile Leu Lys Asn Leu Asn Val			
	100	105 110	
cct ttc gaa gat gtg aat att ctg gag aat gag atg ttg agg caa gga			384
Pro Phe Glu Asp Val Asn Ile Leu Glu Asn Glu Met Leu Arg Gln Gly			
	115	120 125	
ctt aaa gag tat tcg aat tgg ccg acg ttt cct cag ctt tat atc ggc			432
Leu Lys Glu Tyr Ser Asn Trp Pro Thr Phe Pro Gln Leu Tyr Ile Gly			
	130	135 140	
ggt gag ttt ttc ggt ggt tgt gat att act ctt gag gcg ttt aag act			480
Gly Glu Phe Phe Gly Gly Cys Asp Ile Thr Leu Glu Ala Phe Lys Thr			
	145	150 155 160	
gga gaa ttg cag gaa gag gtg gag aaa gct atg tgc tct tga			522
Gly Glu Leu Gln Glu Glu Val Glu Lys Ala Met Cys Ser			
	165	170	

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20 25 30

Leu Lys Pro Thr Ser Ala Leu Val Val His Asn His Gln Leu Ser Phe  
35 40 45

Tyr Gly Ser Asn Leu Lys Leu Lys Pro Thr Lys Phe Arg Cys Ser Ala  
50 55 60

Ser Ala Leu Thr Pro Gln Leu Lys Asp Thr Leu Glu Lys Leu Val Asn  
65 70 75 80

Ser Glu Lys Val Val Leu Phe Met Lys Gly Thr Arg Asp Phe Pro Met  
85 90 95

Cys Gly Phe Ser Asn Thr Val Val Gln Ile Leu Lys Asn Leu Asn Val  
 100 105 110

Pro Phe Glu Asp Val Asn Ile Leu Glu Asn Glu Met Leu Arg Gln Gly  
 115 120 125

Leu Lys Glu Tyr Ser Asn Trp Pro Thr Phe Pro Gln Leu Tyr Ile Gly  
 130 135 140

Gly Glu Phe Phe Gly Gly Cys Asp Ile Thr Leu Glu Ala Phe Lys Thr  
 145 150 155 160

Gly Glu Leu Gln Glu Glu Val Glu Lys Ala Met Cys Ser  
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 Arg Val Val Arg Pro His Val Ser Arg Asn Thr Pro Val Ile Thr Leu  
 20 25 30  
 tat tca cgc ttc aca cca tcc ttc tcc ttc cca tct ctc tcc ttc aca 144  
 Tyr Ser Arg Phe Thr Pro Ser Phe Ser Phe Pro Ser Leu Ser Phe Thr  
 35 40 45  
 ctc cgt gac aca gct ccg tct cgt cgt cgt tcc ttc ttt atc gcc tcc 192  
 Leu Arg Asp Thr Ala Pro Ser Arg Arg Arg Ser Phe Phe Ile Ala Ser  
 50 55 60  
 gcc gtc aaa tct cta acg gag acg gag ctg ctt cca atc aca gag gct 240  
 Ala Val Lys Ser Leu Thr Glu Thr Glu Leu Leu Pro Ile Thr Glu Ala  
 65 70 75 80  
 gat tca atc ccg tcc gct tcc ggt gta tac gct gta tac gat aag agc 288  
 Asp Ser Ile Pro Ser Ala Ser Gly Val Tyr Ala Val Tyr Asp Lys Ser  
 85 90 95  
 gac gag ctt cag ttc gtc gga att tct cgg aac atc gct gcg agt gtc 336  
 Asp Glu Leu Gln Phe Val Gly Ile Ser Arg Asn Ile Ala Ala Ser Val  
 100 105 110  
 tct gct cat ctc aaa tct gtg ccg gag ctt tgt ggc tcc gtt aag gtt 384  
 Ser Ala His Leu Lys Ser Val Pro Glu Leu Cys Gly Ser Val Lys Val  
 115 120 125

gga ata gta gaa gaa cca gat aaa gca gtt tta aca caa gca tgg aaa 432  
 Gly Ile Val Glu Glu Pro Asp Lys Ala Val Leu Thr Gln Ala Trp Lys  
 130 135 140

tta tgg ata gaa gaa cat ata aaa gta act gga aaa gtt ccg ccg ggg 480  
 Leu Trp Ile Glu Glu His Ile Lys Val Thr Gly Lys Val Pro Pro Gly  
 145 150 155 160

aat aag tca ggg aac aac aca ttt gtc aaa caa act ccg agg aag aaa 528  
 Asn Lys Ser Gly Asn Asn Thr Phe Val Lys Gln Thr Pro Arg Lys Lys  
 165 170 175

tcc gat atc cgt ctc act cca ggt cgc cat gtt gag ctc acg gtt cct 576  
 Ser Asp Ile Arg Leu Thr Pro Gly Arg His Val Glu Leu Thr Val Pro  
 180 185 190

ttg gag gaa ctg att gac cgt tta gtg aaa gag agc aaa gtg gta gct 624  
 Leu Glu Glu Leu Ile Asp Arg Leu Val Lys Glu Ser Lys Val Val Ala  
 195 200 205

ttc ata aaa gga tca agg agt gct cct caa tgt gga ttc tca cag aga 672  
 Phe Ile Lys Gly Ser Arg Ser Ala Pro Gln Cys Gly Phe Ser Gln Arg  
 210 215 220

gtt gtt ggg att ctt gaa agc caa gga gtt gat tat gaa act gtt gat 720  
 Val Val Gly Ile Leu Glu Ser Gln Gly Val Asp Tyr Glu Thr Val Asp  
 225 230 235 240

gtt ctt gac gat gag tat aat cat ggg cta agg gag acg ctt aag aac 768  
 Val Leu Asp Asp Glu Tyr Asn His Gly Leu Arg Glu Thr Leu Lys Asn  
 245 250 255

tac agc aat tgg cca acg ttt cca cag ata ttt gtg aaa gga gaa ctt 816  
 Tyr Ser Asn Trp Pro Thr Phe Pro Gln Ile Phe Val Lys Gly Glu Leu  
 260 265 270

gta gga gga tgt gat att ttg acc tca atg tat gaa aat ggt gaa ctt 864  
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 275 280 285

gcc aat atc ttg aac tag 882  
 Ala Asn Ile Leu Asn  
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 20 25 30

Tyr Ser Arg Phe Thr Pro Ser Phe Ser Phe Pro Ser Leu Ser Phe Thr  
 35 40 45

Leu Arg Asp Thr Ala Pro Ser Arg Arg Arg Ser Phe Phe Ile Ala Ser  
50 55 60

Ala Val Lys Ser Leu Thr Glu Thr Glu Leu Leu Pro Ile Thr Glu Ala  
65 70 75 80

Asp Ser Ile Pro Ser Ala Ser Gly Val Tyr Ala Val Tyr Asp Lys Ser  
85 90 95

Asp Glu Leu Gln Phe Val Gly Ile Ser Arg Asn Ile Ala Ala Ser Val  
100 105 110

Ser Ala His Leu Lys Ser Val Pro Glu Leu Cys Gly Ser Val Lys Val  
115 120 125

Gly Ile Val Glu Glu Pro Asp Lys Ala Val Leu Thr Gln Ala Trp Lys  
130 135 140

Leu Trp Ile Glu Glu His Ile Lys Val Thr Gly Lys Val Pro Pro Gly  
145 150 155 160

Asn Lys Ser Gly Asn Asn Thr Phe Val Lys Gln Thr Pro Arg Lys Lys  
165 170 175

Ser Asp Ile Arg Leu Thr Pro Gly Arg His Val Glu Leu Thr Val Pro  
180 185 190

Leu Glu Glu Leu Ile Asp Arg Leu Val Lys Glu Ser Lys Val Val Ala  
195 200 205

Phe Ile Lys Gly Ser Arg Ser Ala Pro Gln Cys Gly Phe Ser Gln Arg  
210 215 220

Val Val Gly Ile Leu Glu Ser Gln Gly Val Asp Tyr Glu Thr Val Asp  
225 230 235 240

Val Leu Asp Asp Glu Tyr Asn His Gly Leu Arg Glu Thr Leu Lys Asn  
245 250 255

Tyr Ser Asn Trp Pro Thr Phe Pro Gln Ile Phe Val Lys Gly Glu Leu  
260 265 270

Val Gly Gly Cys Asp Ile Leu Thr Ser Met Tyr Glu Asn Gly Glu Leu  
275 280 285

Ala Asn Ile Leu Asn  
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 ttg cgc cac agc gga gca cca ctc gtg ctt cac ttc tgg gct tct tgg 96  
 Leu Arg His Ser Gly Ala Pro Leu Val Leu His Phe Trp Ala Ser Trp  
 20 25 30  
 tgt gac gct tcg aag cag atg gat caa gtt ttc tct cat ctc gct act 144  
 Cys Asp Ala Ser Lys Gln Met Asp Gln Val Phe Ser His Leu Ala Thr  
 35 40 45  
 gat ttc cct cgt gct cac ttc ttt agg gta gaa gct gag gaa cat cct 192  
 Asp Phe Pro Arg Ala His Phe Phe Arg Val Glu Ala Glu Glu His Pro  
 50 55 60  
 gag ata tct gag gct tat tct gtt gct ctt gtg ccg tat ttc gtc ttc 240  
 Glu Ile Ser Glu Ala Tyr Ser Val Ala Leu Val Pro Tyr Phe Val Phe  
 65 70 75 80  
 ttc aag gat ggc aaa act gtg gat aca ctt gaa ggg gca gat cca tca 288  
 Phe Lys Asp Gly Lys Thr Val Asp Thr Leu Glu Gly Ala Asp Pro Ser  
 85 90 95  
 agt tta gct aat aaa gtt ggc aaa gtt gct ggt tct att act cct gca 336  
 Ser Leu Ala Asn Lys Val Gly Lys Val Ala Gly Ser Ile Thr Pro Ala  
 100 105 110  
 agc tta ggg ttg gct gca ggg cca acg att ctt gaa act gtt aag aag 384  
 Ser Leu Gly Leu Ala Ala Gly Pro Thr Ile Leu Glu Thr Val Lys Lys  
 115 120 125  
 aat gcg aaa gct tct gga caa gac cga gct cag cct gta tct acc gct 432  
 Asn Ala Lys Ala Ser Gly Gln Asp Arg Ala Gln Pro Val Ser Thr Ala  
 130 135 140  
 gat gct ctc aag aat cgt ttg gaa aaa ctc acc ctg tta tgt tat tca 480  
 Asp Ala Leu Lys Asn Arg Leu Glu Lys Leu Thr Leu Leu Cys Tyr Ser  
 145 150 155 160  
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 20 25 30

Cys Asp Ala Ser Lys Gln Met Asp Gln Val Phe Ser His Leu Ala Thr  
 35 40 45

Asp Phe Pro Arg Ala His Phe Phe Arg Val Glu Ala Glu Glu His Pro  
 50 55 60

Glu Ile Ser Glu Ala Tyr Ser Val Ala Leu Val Pro Tyr Phe Val Phe  
 65 70 75 80

Phe Lys Asp Gly Lys Thr Val Asp Thr Leu Glu Gly Ala Asp Pro Ser  
 85 90 95

Ser Leu Ala Asn Lys Val Gly Lys Val Ala Gly Ser Ile Thr Pro Ala  
 100 105 110

Ser Leu Gly Leu Ala Ala Gly Pro Thr Ile Leu Glu Thr Val Lys Lys  
 115 120 125

Asn Ala Lys Ala Ser Gly Gln Asp Arg Ala Gln Pro Val Ser Thr Ala  
 130 135 140

Asp Ala Leu Lys Asn Arg Leu Glu Lys Leu Thr Leu Leu Cys Tyr Ser  
 145 150 155 160

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ttg cgc cag agc ggc gca cca gtc gtg ctt cac ttc tgg gct tct tgg 96  
 Leu Arg Gln Ser Gly Ala Pro Val Val Leu His Phe Trp Ala Ser Trp  
 20 25 30

tgt gat gct tcg aag cag atg gat caa gtt ttc tct cat ctc gct act 144  
 Cys Asp Ala Ser Lys Gln Met Asp Gln Val Phe Ser His Leu Ala Thr  
 35 40 45

gat ttc cct cgt gct cac ttc ttt agg gtt gaa gct gag gaa cat cct 192  
 Asp Phe Pro Arg Ala His Phe Phe Arg Val Glu Ala Glu Glu His Pro  
 50 55 60

gag ata tct gag gct tac tct gtt gct gct gtg cct tat ttc gtc ttc Glu Ile Ser Glu Ala Tyr Ser Val Ala Ala Val Pro Tyr Phe Val Phe 65 70 75 80	240
ttc aag gat ggt aaa act gtg gat aca ctt gag ggt gca gat cca tca Phe Lys Asp Gly Lys Thr Val Asp Thr Leu Glu Gly Ala Asp Pro Ser 85 90 95	288
agt tta gct aat aag gtt ggc aaa gtt gct ggt tct agt act tct gcg Ser Leu Ala Asn Lys Val Gly Lys Val Ala Gly Ser Ser Thr Ser Ala 100 105 110	336
gag cct gct gct cct gca agc tta ggg ttg gct gct ggg cca acg att Glu Pro Ala Ala Pro Ala Ser Leu Gly Leu Ala Ala Gly Pro Thr Ile 115 120 125	384
ctt gaa act gtg aag gag aat gcg aaa gct tct tta caa gac cga gct Leu Glu Thr Val Lys Glu Asn Ala Lys Ala Ser Leu Gln Asp Arg Ala 130 135 140	432
cag cct gta tct acc gcc gat gct ctc aag agc cgt ttg gaa aag ctc Gln Pro Val Ser Thr Ala Asp Ala Leu Lys Ser Arg Leu Glu Lys Leu 145 150 155 160	480
act aat tct cac cct gtc atg tta ttc atg aaa ggt att cct gaa gag Thr Asn Ser His Pro Val Met Leu Phe Met Lys Gly Ile Pro Glu Glu 165 170 175	528
cct agg tgt ggg ttt agc agg aaa gta gtt gac att ttg aaa gag gtt Pro Arg Cys Gly Phe Ser Arg Lys Val Val Asp Ile Leu Lys Glu Val 180 185 190	576
aac gtt gat ttt gga agt ttt gac ata cta tcg gat aac gaa gtg cga Asn Val Asp Phe Gly Ser Phe Asp Ile Leu Ser Asp Asn Glu Val Arg 195 200 205	624
gag ggt ttg aag aaa ttc tct aac tgg cca acg ttt cct cag ctg tac Glu Gly Leu Lys Lys Phe Ser Asn Trp Pro Thr Phe Pro Gln Leu Tyr 210 215 220	672
tgc aac gga gag ctt ctt ggt gga gct gat atc gca ata gcg atg cac Cys Asn Gly Glu Leu Leu Gly Gly Ala Asp Ile Ala Ile Ala Met His 225 230 235 240	720
gag agc ggt gaa cta aaa gat gct ttc aaa gat ctt ggg atc acg aca Glu Ser Gly Glu Leu Lys Asp Ala Phe Lys Asp Leu Gly Ile Thr Thr 245 250 255	768
gtt ggt tca aaa gaa agt cag gat gaa gct gga aaa gga gga ggg gtt Val Gly Ser Lys Glu Ser Gln Asp Glu Ala Gly Lys Gly Gly Gly Val 260 265 270	816
agt tca gga aac aca ggc tta agt gag acc ctc cga gct cgg ctc gaa Ser Ser Gly Asn Thr Gly Leu Ser Glu Thr Leu Arg Ala Arg Leu Glu 275 280 285	864
ggt ctg gtc aat tcc aaa cca gtt atg ctg ttc atg aaa gga aga cca Gly Leu Val Asn Ser Lys Pro Val Met Leu Phe Met Lys Gly Arg Pro 290 295 300	912
gaa gaa cca aag tgt ggg ttc agt ggg aaa gtg gtt gaa atc ctc aac Glu Glu Pro Lys Cys Gly Phe Ser Gly Lys Val Val Glu Ile Leu Asn	960



305	310	315	320	
caa gaa aaa atc gag ttt ggg agt ttc gat atc ctc tta gat gac gaa				1008
Gln Glu Lys Ile Glu Phe Gly Ser Phe Asp Ile Leu Leu Asp Asp Glu				
325		330	335	
gtt cgc caa ggc ctt aaa gtg tat tca aac tgg tca agc tat cct cag				1056
Val Arg Gln Gly Leu Lys Val Tyr Ser Asn Trp Ser Ser Tyr Pro Gln				
340		345	350	
ctt tac gtg aaa ggc gag ctt atg ggt gga tca gac att gtc ttg gag				1104
Leu Tyr Val Lys Gly Glu Leu Met Gly Gly Ser Asp Ile Val Leu Glu				
355		360	365	
atg caa aag agc ggt gag ctg aaa aag gtc ttg acc gag aaa ggg atc				1152
Met Gln Lys Ser Gly Glu Leu Lys Lys Val Leu Thr Glu Lys Gly Ile				
370		375	380	
act gga gaa cag agt ctt gaa gat aga ttg aag gca ctg atc aat tcc				1200
Thr Gly Glu Gln Ser Leu Glu Asp Arg Leu Lys Ala Leu Ile Asn Ser				
385		390	395	400
tcg gaa gta atg cta ttc atg aaa ggt tca cca gat gaa ccg aaa tgc				1248
Ser Glu Val Met Leu Phe Met Lys Gly Ser Pro Asp Glu Pro Lys Cys				
405		410	415	
gga ttt agc tcc aaa gtt gtg aaa gca ttg aga gga gaa aac gtg agt				1296
Gly Phe Ser Ser Lys Val Val Lys Ala Leu Arg Gly Glu Asn Val Ser				
420		425	430	
ttc gga tcg ttt gat atc ttg act gat gaa gaa gta agg caa ggg att				1344
Phe Gly Ser Phe Asp Ile Leu Thr Asp Glu Glu Val Arg Gln Gly Ile				
435		440	445	
aag aat ttc tca aac tgg cca act ttt cct cag cta tac tac aaa ggt				1392
Lys Asn Phe Ser Asn Trp Pro Thr Phe Pro Gln Leu Tyr Tyr Lys Gly				
450		455	460	
gag tta att gga gga tgt gat atc att atg gag cta agt gag agt ggt				1440
Glu Leu Ile Gly Gly Cys Asp Ile Ile Met Glu Leu Ser Glu Ser Gly				
465		470	475	480
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Asp Leu Lys Ala Thr Leu Ser Glu				
485				

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20 25 30

Cys Asp Ala Ser Lys Gln Met Asp Gln Val Phe Ser His Leu Ala Thr  
35 40 45

Asp Phe Pro Arg Ala His Phe Phe Arg Val Glu Ala Glu Glu His Pro  
50 55 60

Glu Ile Ser Glu Ala Tyr Ser Val Ala Ala Val Pro Tyr Phe Val Phe  
65 70 75 80

Phe Lys Asp Gly Lys Thr Val Asp Thr Leu Glu Gly Ala Asp Pro Ser  
85 90 95

Ser Leu Ala Asn Lys Val Gly Lys Val Ala Gly Ser Ser Thr Ser Ala  
100 105 110

Glu Pro Ala Ala Pro Ala Ser Leu Gly Leu Ala Ala Gly Pro Thr Ile  
115 120 125

Leu Glu Thr Val Lys Glu Asn Ala Lys Ala Ser Leu Gln Asp Arg Ala  
130 135 140

Gln Pro Val Ser Thr Ala Asp Ala Leu Lys Ser Arg Leu Glu Lys Leu  
145 150 155 160

Thr Asn Ser His Pro Val Met Leu Phe Met Lys Gly Ile Pro Glu Glu  
165 170 175

Pro Arg Cys Gly Phe Ser Arg Lys Val Val Asp Ile Leu Lys Glu Val  
180 185 190

Asn Val Asp Phe Gly Ser Phe Asp Ile Leu Ser Asp Asn Glu Val Arg  
195 200 205

Glu Gly Leu Lys Lys Phe Ser Asn Trp Pro Thr Phe Pro Gln Leu Tyr  
210 215 220

Cys Asn Gly Glu Leu Leu Gly Gly Ala Asp Ile Ala Ile Ala Met His  
225 230 235 240

Glu Ser Gly Glu Leu Lys Asp Ala Phe Lys Asp Leu Gly Ile Thr Thr  
245 250 255

Val Gly Ser Lys Glu Ser Gln Asp Glu Ala Gly Lys Gly Gly Gly Val  
260 265 270

Ser Ser Gly Asn Thr Gly Leu Ser Glu Thr Leu Arg Ala Arg Leu Glu  
275 280 285

Gly Leu Val Asn Ser Lys Pro Val Met Leu Phe Met Lys Gly Arg Pro

290                      295                      300  
 Glu Glu Pro Lys Cys Gly Phe Ser Gly Lys Val Val Glu Ile Leu Asn  
 305                      310                      315                      320  
 Gln Glu Lys Ile Glu Phe Gly Ser Phe Asp Ile Leu Leu Asp Asp Glu  
                     325                      330                      335  
 Val Arg Gln Gly Leu Lys Val Tyr Ser Asn Trp Ser Ser Tyr Pro Gln  
                     340                      345                      350  
 Leu Tyr Val Lys Gly Glu Leu Met Gly Gly Ser Asp Ile Val Leu Glu  
                     355                      360                      365  
 Met Gln Lys Ser Gly Glu Leu Lys Lys Val Leu Thr Glu Lys Gly Ile  
                     370                      375                      380  
 Thr Gly Glu Gln Ser Leu Glu Asp Arg Leu Lys Ala Leu Ile Asn Ser  
 385                      390                      395                      400  
 Ser Glu Val Met Leu Phe Met Lys Gly Ser Pro Asp Glu Pro Lys Cys  
                     405                      410                      415  
 Gly Phe Ser Ser Lys Val Val Lys Ala Leu Arg Gly Glu Asn Val Ser  
                     420                      425                      430  
 Phe Gly Ser Phe Asp Ile Leu Thr Asp Glu Glu Val Arg Gln Gly Ile  
                     435                      440                      445  
 Lys Asn Phe Ser Asn Trp Pro Thr Phe Pro Gln Leu Tyr Tyr Lys Gly  
                     450                      455                      460  
 Glu Leu Ile Gly Gly Cys Asp Ile Ile Met Glu Leu Ser Glu Ser Gly  
 465                      470                      475                      480  
 Asp Leu Lys Ala Thr Leu Ser Glu  
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atccggtctc gctatctctc ttccgcatct cgggacgcg atg gcg gcg ctg ctg 114  
Met Ala Ala Leu Leu  
1 5

ggc cgg agg ttc ggg atg gcg gcg gcg gcg ctc atc gcc ctc gcg gcg 162  
Gly Arg Arg Phe Gly Met Ala Ala Ala Ala Leu Ile Ala Leu Ala Ala  
10 15 20

ctc gga tcc gcc gcc tcg ggg acg gcg tcc aag tcg tcc ttc gtg aaa 210  
Leu Gly Ser Ala Ala Ser Gly Thr Ala Ser Lys Ser Ser Phe Val Lys  
25 30 35

tcc acc gtc aaa gcc cac gac gtc gtc ata ttc tcc aag tca tac tgc 258  
Ser Thr Val Lys Ala His Asp Val Val Ile Phe Ser Lys Ser Tyr Cys  
40 45 50

ccg tac tgt aga aga gcc aaa gct gtg ttc aag gaa ctt gaa ctg aag 306  
Pro Tyr Cys Arg Arg Ala Lys Ala Val Phe Lys Glu Leu Glu Leu Lys  
55 60 65

aag gag ccg tat gtt gtg gag ctt gat caa cga gag gat ggt tgg gag 354  
Lys Glu Pro Tyr Val Val Glu Leu Asp Gln Arg Glu Asp Gly Trp Glu  
70 75 80 85

att cag gat gcc tta tct gac atg gtt ggc agg cga act gtt cct caa 402  
Ile Gln Asp Ala Leu Ser Asp Met Val Gly Arg Arg Thr Val Pro Gln  
90 95 100

gtt ttt gtc cat ggg aag cac ctg ggt ggc tct gat gat act gtt gaa 450  
Val Phe Val His Gly Lys His Leu Gly Gly Ser Asp Asp Thr Val Glu  
105 110 115

gca tat gag agt ggc aag cta gcc aaa ctt ttg aac att gat gtc aaa 498  
Ala Tyr Glu Ser Gly Lys Leu Ala Lys Leu Leu Asn Ile Asp Val Lys  
120 125 130

gaa gat ctt tgagtagtaa tagtttagca tcaatggcag gcctctttca 547  
Glu Asp Leu  
135

tttccataga acatacccaa atactatgca actatgaatt cttcatagaa tttggctgtg 607

aatgtctctt ttagccctt t 628

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1 5 10 15

Ile Ala Leu Ala Ala Leu Gly Ser Ala Ala Ser Gly Thr Ala Ser Lys  
20 25 30

Ser Ser Phe Val Lys Ser Thr Val Lys Ala His Asp Val Val Ile Phe  
35 40 45

Ser Lys Ser Tyr Cys Pro Tyr Cys Arg Arg Ala Lys Ala Val Phe Lys  
50 55 60

Glu Leu Glu Leu Lys Lys Glu Pro Tyr Val Val Glu Leu Asp Gln Arg  
65 70 75 80

Glu Asp Gly Trp Glu Ile Gln Asp Ala Leu Ser Asp Met Val Gly Arg  
85 90 95

Arg Thr Val Pro Gln Val Phe Val His Gly Lys His Leu Gly Gly Ser  
100 105 110

Asp Asp Thr Val Glu Ala Tyr Glu Ser Gly Lys Leu Ala Lys Leu Leu  
115 120 125

Asn Ile Asp Val Lys Glu Asp Leu  
130 135

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<211> 733  
<212> DNA  
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Met Gly Ile Ala Ser Ser Ser Ser  
1 5

acc ccg gaa tcc agg aag atg gcg ctc gcc aag gcc aag gag acc gtc 160  
Thr Pro Glu Ser Arg Lys Met Ala Leu Ala Lys Ala Lys Glu Thr Val  
10 15 20 25

gcc tcc gct ccc gtc gtc gtc tac agc aag tct tac tgt cct ttt tgc 208  
Ala Ser Ala Pro Val Val Val Tyr Ser Lys Ser Tyr Cys Pro Phe Cys  
30 35 40

gtc cgt gtg aag aag ttg ttc gag cag ctt gga gca act ttc aag gcc 256  
Val Arg Val Lys Lys Leu Phe Glu Gln Leu Gly Ala Thr Phe Lys Ala  
45 50 55

att gag ttg gat ggg gag agt gat gga tct gag ctg cag tcg gca ctt 304  
Ile Glu Leu Asp Gly Glu Ser Asp Gly Ser Glu Leu Gln Ser Ala Leu  
60 65 70

gct gaa tgg act gga caa agg act gtt cca aat gtc ttc atc aat ggg 352  
Ala Glu Trp Thr Gly Gln Arg Thr Val Pro Asn Val Phe Ile Asn Gly  
75 80 85

aag cat att ggt ggc tgt gat gat act ttg gca ttg aac aat gaa ggg 400  
Lys His Ile Gly Gly Cys Asp Asp Thr Leu Ala Leu Asn Asn Glu Gly  
90 95 100 105

aag ctg gtg cct ctg ctg acc gag gct gga gca att gcc agt tct gca 448  
 Lys Leu Val Pro Leu Leu Thr Glu Ala Gly Ala Ile Ala Ser Ser Ala  
                   110                  115                  120

aag acg aca atc acc gca tagttcttcg tgggacactg ggactagcct 496  
 Lys Thr Thr Ile Thr Ala  
                   125

tcgttgacct ctttatactg catccattct attagataat aaaggtggat gtttgtttgg 556

caagaccatt acttgttgcc gtctagtatc gtgtgatagc tatcctgtgc ccgtgtgaaa 616

ctccttggac atcaataata tcgtctttgt gatagcagtt cgctgaaaaa aaaaaaaaaa 676

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa 733

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Ala Leu Ala Lys Ala Lys Glu Thr Val Ala Ser Ala Pro Val Val Val  
                   20                  25                  30

Tyr Ser Lys Ser Tyr Cys Pro Phe Cys Val Arg Val Lys Lys Leu Phe  
           35                  40                  45

Glu Gln Leu Gly Ala Thr Phe Lys Ala Ile Glu Leu Asp Gly Glu Ser  
       50                  55                  60

Asp Gly Ser Glu Leu Gln Ser Ala Leu Ala Glu Trp Thr Gly Gln Arg  
 65                  70                  75                  80

Thr Val Pro Asn Val Phe Ile Asn Gly Lys His Ile Gly Gly Cys Asp  
           85                  90                  95

Asp Thr Leu Ala Leu Asn Asn Glu Gly Lys Leu Val Pro Leu Leu Thr  
           100                  105                  110

Glu Ala Gly Ala Ile Ala Ser Ser Ala Lys Thr Thr Ile Thr Ala  
       115                  120                  125

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 <222> (92)..(586)

&lt;223&gt; OZ1116C26232

&lt;400&gt; 49

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ccggcgatcc ctccatcccc ctccatcacc a atg ccg ccg cgg agc ctc acc 112
                               Met Pro Pro Arg Ser Leu Thr
                               1                               5

ctc tcc cgc ctt ccc gtg gcc gcc ctt ggc ctc ccc ttc tct tct tgc 160
Leu Ser Arg Leu Pro Val Ala Ala Leu Gly Leu Pro Phe Ser Ser Cys
                               10                               15                               20

tcc ccg cct cct cct cgc ctt cgc ttc ccc ttc gcc gca cgc cgc gcc 208
Ser Pro Pro Pro Pro Arg Leu Arg Phe Pro Phe Ala Ala Arg Arg Ala
                               25                               30                               35

agg tcc ctc gcc acc agg gcc tcc tcc tcc tct ccg gat tcc tcc ttc 256
Arg Ser Leu Ala Thr Arg Ala Ser Ser Ser Ser Pro Asp Ser Ser Phe
40                               45                               50                               55

ggc tcg cgg atg gag gac tct gtc aag agg acg ctc gcc gac aac ccc 304
Gly Ser Arg Met Glu Asp Ser Val Lys Arg Thr Leu Ala Asp Asn Pro
                               60                               65                               70

gtc gtc atc tac tcc aag tcc tgg tgc tcc tac tcc atg gag gtc aag 352
Val Val Ile Tyr Ser Lys Ser Trp Cys Ser Tyr Ser Met Glu Val Lys
75                               80                               85

gcg ctc ttc aag cgg atc gcc gtc cag ccg cac gtc atc gag ctc gac 400
Ala Leu Phe Lys Arg Ile Gly Val Gln Pro His Val Ile Glu Leu Asp
90                               95                               100

caa ctc gcc gca cag gga cct cag ttg caa aag gtg tta gag cgg ctg 448
Gln Leu Gly Ala Gln Gly Pro Gln Leu Gln Lys Val Leu Glu Arg Leu
105                               110                               115

act gga cag tcc act gtt cct aat gtt ttc att ggt gga aag cac att 496
Thr Gly Gln Ser Thr Val Pro Asn Val Phe Ile Gly Gly Lys His Ile
120                               125                               130                               135

ggt gcc tgt aca gat act gtg aag ctt cat cgc aaa ggg gag cta gct 544
Gly Gly Cys Thr Asp Thr Val Lys Leu His Arg Lys Gly Glu Leu Ala
140                               145                               150

acc atg ctg tca gag ctg gat atc gac gtc aac aac tca tga 586
Thr Met Leu Ser Glu Leu Asp Ile Asp Val Asn Asn Ser
155                               160

caacattgaa catggtttgc tatactggat atctgaggtt tcaatgactt gagcagtcgt 646
gtaatgagat ttgttagcca tgtttactaa ttcaatgcac attttatgta accgcttccc 706
ttgatcagct accgaatttt gactaatgtg tatccaccgg cgaacttg 754

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&lt;210&gt; 50

&lt;211&gt; 164

&lt;212&gt; PRT

&lt;213&gt; Oryza sativa

&lt;400&gt; 50

Met Pro Pro Arg Ser Leu Thr Leu Ser Arg Leu Pro Val Ala Ala Leu

1

5

10

15

Gly Leu Pro Phe Ser Ser Cys Ser Pro Pro Pro Pro Arg Leu Arg Phe  
 20 25 30

Pro Phe Ala Ala Arg Arg Ala Arg Ser Leu Ala Thr Arg Ala Ser Ser  
 35 40 45

Ser Ser Pro Asp Ser Ser Phe Gly Ser Arg Met Glu Asp Ser Val Lys  
 50 55 60

Arg Thr Leu Ala Asp Asn Pro Val Val Ile Tyr Ser Lys Ser Trp Cys  
 65 70 75 80

Ser Tyr Ser Met Glu Val Lys Ala Leu Phe Lys Arg Ile Gly Val Gln  
 85 90 95

Pro His Val Ile Glu Leu Asp Gln Leu Gly Ala Gln Gly Pro Gln Leu  
 100 105 110

Gln Lys Val Leu Glu Arg Leu Thr Gly Gln Ser Thr Val Pro Asn Val  
 115 120 125

Phe Ile Gly Gly Lys His Ile Gly Gly Cys Thr Asp Thr Val Lys Leu  
 130 135 140

His Arg Lys Gly Glu Leu Ala Thr Met Leu Ser Glu Leu Asp Ile Asp  
 145 150 155 160

Val Asn Asn Ser

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 <223> primer

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44

<210> 52  
 <211> 46  
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<222> (1)..(46)

<223> primer

<400> 52

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46

<210> 53

<211> 44

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46

<210> 55

<211> 38

<212> DNA

<213> Artificial

<220>

<221> cloningcassette

<222> (1)..(38)

<223> cloning cassette

<400> 55

ggaattccag ctgaccacca tggcaattcc cggggatc

38

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